Laboratory Manual

for CHEMICAL and BACTERIAL

ANALYSIS of WATER and SEWAGE

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by

FRANK R. THEROUX, M.C.E.
Professor of Civil Engineering
Michigan State College

EDWARD F. ELDRIDGE, M.S.
Engineering Research Associate
Michigan State College

W. LeROY MALLMANN, Ph.D.
Professor of Bacteriology
Michigan State College

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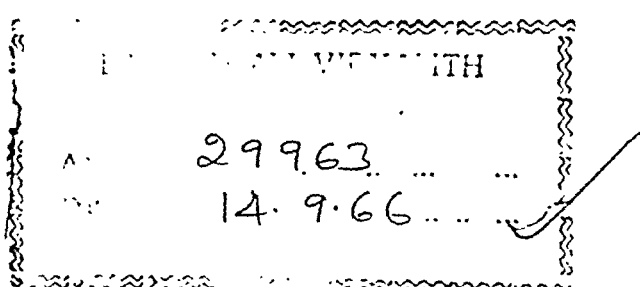

PREFACE TO THE THIRD EDITION

A revision of this manual is considered desirable at this time since new methods of testing are available and numerous minor changes have been made in existing methods. A number of new tests and discussions have been added to the chemical sections of the book.

Because many requests have been received for a section dealing with the testing of boiler waters, this section has been added to the manual. The most commonly used tests have been written in the step-by-step form peculiar to this book. The section has been divided in two parts. The first deals with tests used in the field or power plant where a minimum of equipment and personnel is available. The second division gives additional procedures for power laboratory testing which require more equipment and more careful manipulation.

Another feature not previously contained in the discussion is the addition of problems and their answers following a number of the items in Section III. These should be valuable in cases where the manual is used as a text.

Extensive changes have been made in the rewriting of the bacteriological section. Many new developments resulting from recent studies of bacteriological testing have influenced the revision.

The authors again invite criticisms, suggestions and corrections.

FRANK R. THEROUX,
EDWARD F. ELDRIDGE,
W. LEROY MALLMANN.

EAST LANSING, MICHIGAN,
May, 1943.

PREFACE TO THE FIRST EDITION

The authors have long felt the need for a simple and concise laboratory manual of water and sewage chemical analysis and have sought to fill that need in the preparation of this book.

The book contains specific directions, in outline form, for making the chemical determinations necessary for the control of water and sewage treatment plants, the analysis of polluted water and the examination of industrial wastes. Each determination is accompanied by calculation formulas, many of which are numerically illustrated. In addition to the specific directions for the tests, the book contains methods of sampling, laboratory technique, a discussion of the chemistry involved, interpretation of results and related topics, making it more valuable as a general manual for those using the results of water and sewage analysis.

The manual will be useful to:

1. Those engaged in water and sewage treatment plant operation who find it necessary, along with other duties, to make certain laboratory determinations, but who because of lack of training in chemistry must rely upon specific directions in carrying out the steps involved.

2. Those with some training in chemistry, but having only infrequent occasion to make certain laboratory determinations on water, sewage and industrial wastes.

3. The plant chemists who will find the manual a convenient and ready reference.

4. The sanitary engineer engaged in making stream surveys or studying the characteristics of water or sewage in connection with plant design.

5. Colleges and universities offering instruction in water and sewage analysis which will find the manual useful as a laboratory text.

FRANK R. THEROUX,
EDWARD F. ELDRIDGE.

EAST LANSING, MICHIGAN,
February, 1935.

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CHEMICAL ANALYSIS OF WATER AND SEWAGE

SECTION I

METHODS FOR CHEMICAL ANALYSIS

DIVISION I

WATER

Note.—Numbers inclosed in parentheses, thus (3), refer to numbered reagents and solutions in Sec. II wherein instructions for their preparation are given.

Total Solids.

1. Clean a platinum evaporating dish and place it in a 103°C. oven for 1 hr. or, if the loss on ignition determination is also to be made, ignite to low red heat in the muffle furnace or over a burner. (A platinum dish is preferred but others may be used.)
2. Place the dish in a desiccator until cool, then weigh.
3. Place the weighed dish on a steam or water bath.
4. Thoroughly mix the sample and measure 100 ml. (a larger volume may be used) by means of a volumetric flask or pipette.
5. Transfer the sample to the dish, rinse the flask or pipette several times with small portions of distilled water and add the rinsings to the dish. Be sure that all suspended matter is transferred to the dish.
6. After the sample is evaporated, dry the dish and residue in the 103°C. oven for 1 hr., cool in the desiccator and weigh.

Calculations.

$$\frac{\text{Increase in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. total solids}$$

Note.—The results of the tests may be checked by testing duplicate portions as illustrated in the following example:

Example.

Sample portion	No. 1	No. 2
Weight of dish and residue (100 ml.)	48.2982 gm.	43.8646 gm.
Weight of dish	48.2540 gm.	43.8210 gm.
Weight of residue	0.0442 gm.	0.0436 gm.

Total solids No. 1:

$$\frac{0.0442 \times 1{,}000{,}000}{100} = 442 \text{ p.p.m.}$$

Total solids No. 2:

$$\frac{0.0436 \times 1{,}000{,}000}{100} = 436 \text{ p.p.m.}$$

Average = 439 p.p.m.

Loss on Ignition (Volatile Matter) and Fixed Residue.

1. Place the evaporating dish containing the residue from the total-solids determination in the muffle furnace, or ignite over a burner at low red heat until the ash is white or nearly so. Not over 1 hr. should be required.
2. Allow the dish to cool and moisten the ash with a few drops of distilled water. (This is to restore water of hydration and absorbed moisture.)
3. Dry in the 103°C. oven for 1 hr., cool in a desiccator and weigh.

Calculations.

$$\frac{\text{Loss in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. loss on ignition}$$

P.p.m. total solids − p.p.m. loss on ignition = p.p.m. fixed residue

Example.

Sample portion	No. 1	No. 2
Weight of dish and residue (100 ml.)	48.2982 gm.	43.8646 gm.
Weight of dish and ash	48.2891 gm.	43.8551 gm.
Loss in weight	0.0091 gm.	0.0095 gm.

Loss on ignition No. 1:

$$\frac{0.0091 \times 1{,}000{,}000}{100} = 91 \text{ p.p.m.}$$

Loss on ignition No. 2:

$$\frac{0.0095 \times 1{,}000{,}000}{100} = 95 \text{ p.p.m.}$$

Average = 93 p.p.m.

Fixed residue (average) = 439 − 93 = 346 p.p.m.

Note.—Part of the loss on ignition may be due to partial decomposition of mineral matter. Therefore this test is not a measure of organic matter. See discussion on Sludge Ignition, page 181.

Suspended and Dissolved Solids, Gooch Crucible Method.

Reagents: Sec. II (1).

1. Place a Gooch crucible in a suction flask and pour into it about 25 ml. of asbestos emulsion (1).
2. Apply the suction gently until the mat is formed.
3. Wash with distilled water until no fibers of asbestos pass through the filter.
4. Dry the crucible in the 103°C. oven and ignite, in a muffle furnace or over a burner, at a low red heat.
5. Allow the crucible to cool. Moisten the mat with a few drops of distilled water.
6. Dry in the 103°C. oven for 1 hr., cool in a desiccator and weigh. (Step Nos. 4 and 5 may be omitted if the loss on ignition of suspended solids is not to be determined.)
7. Measure 100 ml. (use a larger portion if the suspended matter is low) of the well-mixed water by means of a volumetric flask or pipette.
8. Filter the sample portion through the prepared Gooch crucible and follow with several distilled-water rinsings of the flask. Be sure that all suspended matter has been transferred to the crucible.
9. Dry in the oven at 103°C. for 1 hr., cool in the desiccator and weigh.

Calculations.

$$\frac{\text{Increase in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. suspended solids}$$

P.p.m. total solids − p.p.m. suspended solids = p.p.m. dissolved solids

Example.

Sample portion	No. 1	No. 2
Weight of crucible and solids (500 ml.)	15.6208 gm.	14.0921 gm.
Weight of crucible	15.5726 gm.	14.0420 gm.
Weight of solids	0.0482 gm.	0.0501 gm.

Suspended solids No. 1:

$$\frac{0.0482 \times 1{,}000{,}000}{500} = 96.4 \text{ p.p.m.}$$

Suspended solids No. 2:

$$\frac{0.0501 \times 1{,}000{,}000}{500} = 100.2 \text{ p.p.m.}$$

Average = 98.3 p.p.m.

Dissolved solids = 439 − 98.3 = 340.7 p.p.m.

Loss on Ignition, Suspended and Dissolved Solids, Gooch Crucible Method.

1. Ignite the crucible containing the residue from the suspended-solids determination at low red heat.
2. Allow the dish to cool and moisten the ash with a few drops of distilled water.
3. Dry in the oven at 103°C. for 1 hr., cool in a desiccator and weigh.

Calculations.

$$\frac{\text{Loss in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. loss on ignition of suspended solids}$$

P.p.m. loss on ignition total solids − p.p.m. loss on ignition suspended solids = p.p.m. loss on ignition dissolved solids

Example.

Sample portion	No. 1	No. 2
Weight of crucible and solids (500 ml.)	15.6208 gm.	14.0921 gm.
Weight of crucible and ash	15.6000 gm.	14.0711 gm.
Loss in weight	0.0208 gm.	0.0210 gm

Loss on ignition No. 1:

$$\frac{0.0208 \times 1{,}000{,}000}{500} = 41.6 \text{ p.p.m.}$$

Loss on ignition No. 2:

$$\frac{0.0210 \times 1{,}000{,}000}{500} = 42.0 \text{ p.p.m.}$$

Average = 41.8 p.p.m.

Loss on ignition dissolved solids = 93 − 41.8 = 51.2 p.p.m.

Suspended and Dissolved Solids, Filtration Method.

1. Filter a portion of the water sample through a filter paper.
2. Follow the method given for the total-solids determination using 100 ml. of the above filtrate.

Calculations.

$$\frac{\text{Increase in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. dissolved solids}$$

P.p.m. total solids − p.p.m. dissolved solids = p.p.m. suspended solids

Example.

Sample portion	No. 1	No. 2
Weight of dish and residue (100 ml.)	48.2882 gm.	43.8546 gm.
Weight of dish	48.2540 gm.	43.8210 gm.
Weight of dissolved solids	0.0342 gm.	0.0336 gm.

Dissolved solids No. 1:

$$\frac{0.0342 \times 1{,}000{,}000}{100} = 342 \text{ p.p.m.}$$

Dissolved solids No. 2:

$$\frac{0.0336 \times 1{,}000{,}000}{100} = 336 \text{ p.p.m.}$$

$$\text{Average} = 339 \text{ p.p.m.}$$

Suspended solids = 439 — 339 = 100 p.p.m.

Loss on Ignition, Suspended and Dissolved Solids, Filtration Method.

1. Ignite the dish containing the residue from the dissolved-solids determination at low red heat.
2. Allow the dish to cool and moisten the ash with a few drops of distilled water.
3. Dry in the oven at 103°C. for 1 hr., cool in a desiccator and weigh.

Calculations.

$$\frac{\text{Loss in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. loss on ignition of dissolved solids}$$

P.p.m. loss on ignition total solids — p.p.m. loss on ignition dissolved solids = p.p.m. loss on ignition suspended solids

Example.

Sample portion	No. 1	No. 2
Weight of dish and residue (100 ml.)	48.2882 gm.	43.8546 gm.
Weight of dish and ash	48.2832 gm.	43.8493 gm
Loss in weight	0.0050 gm.	0.0053 gm.

Loss on ignition dissolved solids No. 1:

$$\frac{0.0050 \times 1{,}000{,}000}{100} = 50 \text{ p.p.m.}$$

Loss on ignition dissolved solids No. 2:

$$\frac{0.0053 \times 1{,}000{,}000}{100} = 53 \text{ p.p.m.}$$

Average = 51.5 p.p.m.

Loss on ignition suspended solids = 93 − 51.5 = 41.5 p.p.m.

Alkalinity.

Reagents: Sec, II (2), (3), (4).

Discussion: Sec. III, page 140.

1. Pipette 100 ml. of the sample into one Erlenmeyer flask and the same quantity of distilled water into another.
2. Add 3 drops of phenolphthalein indicator (2) to each.
3. If the sample becomes pink, add 0.02N sulfuric acid (3) from a burette until the pink color just disappears and record the number of ml. of acid used.
4. Add 3 drops of methyl orange indicator (4) to each flask.
5. If the sample becomes yellow, add 0.02N sulfuric acid until the first difference in color is noted when compared with the distilled water. The end point is orange. (Methyl orange is yellow in alkaline solution, orange in neutral and red in acid.) Record the number of ml. of acid used.

Calculations.

Total alkalinity as p.p.m. $CaCO_3$ = total ml. acid × 10

Differentiation of alkalinities due to hydroxide (OH), normal carbonate (CO_3) and bicarbonate (HCO_3) is made as explained below.

Let P = the ml. of 0.02N sulfuric acid used for the titration with phenolphthalein and T = the ml. of the acid used for the total titration (phenolphthalein plus methyl orange).

There are five possible conditions as follows:

1. $P = T$
   Hydroxide (p.p.m.) = $P \times 10$
2. $P > \frac{1}{2}T$
   Hydroxide (p.p.m.) = $(2P - T) \times 10$
   Normal carbonate (p.p.m.) = $2(T - P) \times 10$
3. $P = \frac{1}{2}T$
   Normal carbonate (p.p.m.) = $T \times 10$
4. $P < \frac{1}{2}T$
   Normal carbonate (p.p.m.) = $2P \times 10$
   Bicarbonate (p.p.m.) = $(T - 2P) \times 10$
5. $P = 0$
   Bicarbonate (p.p.m.) = $T \times 10$

All of the above results are in terms of p.p.m. as $CaCO_3$.

Examples.

The five possible alkalinity conditions are illustrated in the five different results of the titrations given in the following table.

ALKALINITY CONDITIONS
(Volume of Sample, 100 Ml.)

Condition	Results of titration Ml. of 0.02N acid		Alkalinities (p.p.m. as $CaCO_3$)		
	P	*T*	Hydroxide (OH)	Carbonate (CO_3)	Bicarbonate (HCO_3)
1	18	18	180	0	0
2	12	18	60	120	0
3	9	18	0	180	0
4	6	18	0	120	60
5	0	18	0	0	180

Acidity.

Reagents: Sec. II (2), (5).
Discussion: Sec. III, page 143.

1. Pipette 100 ml. of the sample into an Erlenmeyer flask.
2. Add 3 drops of phenolphthalein indicator (2).
3. Add 0.02N sodium hydroxide (5) from a burette until the first permanent pink color appears and record the number of ml. of sodium hydroxide used.

Calculations.

Ml. of 0.02N NaOH × 10 = p.p.m. total acidity expressed in terms of $CaCO_3$

Carbon Dioxide (CO_2).

Reagents: Sec. II (2), (6).
Discussion: Sec. III, page 143.

1. Fill a 100-ml. Nessler tube to the mark with the sample.
2. Add 10 drops of phenolphthalein indicator (2).
3. Add N/44 sodium hydroxide (6) from a burette, stirring gently, until a slight permanent pink color appears. Record the number of ml. of sodium hydroxide used.

Calculations.

Ml. of N/44 NaOH × 10 = p.p.m. carbon dioxide (CO_2)

Note.—This test should be made at the time the sample is taken because of the ease with which the carbon dioxide escapes. If this is not possible, the sample bottle should be completely filled and stoppered and the sample kept at a temperature lower than that at the time it was collected.

Hydrogen-ion Concentration (pH).

Discussion: Sec. III, page 144.

There are two general types of apparatus adapted for the determination of hydrogen-ion concentration, the colorimetric and the electrometric.

There are many kinds of colorimetric pH outfits on the market most of which are of equal accuracy and convenience for water analysis. There are two general types, (*a*) those having color disks and (*b*) those having standard color solutions.

The following general method can be applied to almost all of the colorimetric outfits:

1. Place a portion of the sample into each of the two or three tubes provided.
2. To one tube add the designated quantity of indicator solution. (The other tubes do not receive the indicator.)
3. Place the tubes in the comparator in such a manner that the color standards are opposite the tubes not containing the indicator. The color comparison must be made by looking through the same thickness of liquid having the same color and turbidity as the sample.
4. Compare the colors, select and read the pH of the tube having a color nearest that of the sample.

Note.—Electrometric pH meters cover the entire range of pH values. Special directions for their use are furnished by the manufacturers.

Total Hardness, Soap Method.

Reagents: Sec. II (7).

Discussion: Sec. III, page 152.

Note.—Large quantities of free CO_2 interfere with the soap test. CO_2 may be removed, before making the soap test, by adding sodium hydroxide solution as in the carbon dioxide test.

1. Pipette 50 ml. of the sample into a 4- to 8-oz. glass bottle.
2. Add the standard soap solution (7) in small portions at first (0.5 ml.), shaking vigorously after each addition.
3. As the end point is approached, the quantity added should be reduced to 0.1 ml. for each addition.
4. After a permanent lather is produced which will last for 5 min. with the bottle on its side, record the ml. of soap solution used.
5. Continue the addition of small quantities of soap solution. If the lather again disappears, the first end point was false owing to the presence of magnesium salts. (The ml. of soap used to obtain this false end point may be used for calculation of the approximate magnesium hardness by substituting in the formula below.)
6. Continue the addition of the soap solution until the true end point is reached and record the ml. used. If the quantity of soap solution used is greater than about 14 ml., repeat the procedure using a smaller sample diluted to 50 ml. with freshly boiled and cooled distilled water.

Calculations.

The hardness may be obtained (1) by use of the formula:

$$\frac{(\text{Ml. soap solution} - \text{ml. lather factor}) \times 1{,}000}{\text{Ml. sample}} = \text{p.p.m. of total hardness as } CaCO_3,$$

or (2) by direct use of the curve, prepared from standardization of the soap solution with waters of known hardness, as described under Note, Standard Soap Solution, page 108?

Example.

Ml. of sample used	50.0
Ml. of soap solution for lather factor	0.4
Ml. of soap solution for sample	6.2

$$\frac{(6.2 - 0.4) \times 1{,}000}{50} = 116 \text{ p.p.m. total hardness as } CaCO_3$$

Hardness, B and B (Boutron and Boudet) Soap Method.

Reagents: Sec. II (7A).
Discussion: Sec. III, page 152.

Note.—This method for the determination of soap hardness is of sufficient accuracy for approximate or field tests. The method is especially adapted for use with zeolite-softened water. Zero-hardness water should not require more than 4 drops of B and B soap solution in 40 ml. of water to produce a lather.

1. By means of a graduate cylinder, measure 40 ml. of the sample into a 3- or 4-oz. glass bottle.
2. Add the soap solution (7A) by drops from a calibrated dropping bottle. Count the drops as they are added and shake the bottle vigorously between small additions of the soap solution.
3. Continue the addition of the soap solution until a lather remains unbroken for 5 min. over the entire surface of the water while the bottle lies on its side. The quantity added between each interval depends upon the hardness of the water. If the water is soft, the mixture should be shaken between each drop. (In order to avoid mistaking the false, or magnesium, end point for the true one, record the number of drops used on appearance of lather and then continue the addition of soap solution until the true end point is definitely reached.)
4. Record the total number of drops of soap solution required.

Calculations.

$$\frac{(\text{Drops of B and B solution required} - 3)}{\text{Drops to make 1 ml.}} \times 5.5 = \text{g.p.g. hardness as } CaCO_3$$

Total Hardness, Soda-reagent Method.

Reagents: Sec. II (3), (4), (8).
Discussion: Sec. III, page 152.

1. Pipette 200 ml. of the sample into a 500-ml. Erlenmeyer flask.
2. Add 3 drops of methyl orange (4).
3. Add 0.02N sulfuric acid (3) from a burette until the first permanent color change is observed.
4. Place 200 ml. of distilled water into a second flask.
5. Boil each for 5 min.
6. Add exactly 25 ml. of soda reagent (8) to each flask and boil for 10 min. until the volume is reduced to about 150 ml.
7. Cool and pour into 200-ml. volumetric flasks.
8. Rinse the solutions into the volumetric flasks with small quantities of hot distilled water.
9. Make up to the mark with boiled distilled water and mix.

10. Filter each solution, rejecting the first 50 ml. of each filtrate.
11. Pipette a 50-ml. portion of each filtrate into an Erlenmeyer flask, add 3 drops of methyl orange and titrate each portion with 0.02N sulfuric acid. Record the ml. of acid used.

Calculations.

(Ml. 0.02N H_2SO_4 used for distilled water − ml. of 0.02N H_2SO_4 used for sample in Step No. 11) × 20 = p.p.m. total hardness as $CaCO_3$

Example.

Ml. of 0.02N H_2SO_4 used for distilled water	31.2
Ml. of 0.02N H_2SO_4 used for sample in Step No. 11	19.6
Difference	11.6

11.6 × 20 = 232 p.p.m. total hardness as $CaCO_3$

Total Hardness, from Mineral Analysis.

Discussion: Sec. III, pages 152 and 160.

Use the methods for the determination of calcium and magnesium (iron and aluminum if present in large amounts) given on pages 18 to 20.

Calculations.

(P.p.m. Ca × 2.496) + (p.p.m. Mg × 4.115) = p.p.m. total hardness as $CaCO_3$

Note.—If iron and aluminum are present in any considerable quantity, they must be determined and the values changed to terms of $CaCO_3$. These results must then be added to the hardness as determined above. To change Fe_2O_3 to $CaCO_3$ multiply by 1.872. To change Al_2O_3 to $CaCO_3$ multiply by 2.932.

Carbonate and Noncarbonate Hardness.

The carbonate and noncarbonate hardness may be calculated from the results of the alkalinity and total hardness determinations.

Calculations.

Carbonate hardness.

Let p.p.m. normal carbonate alkalinity + p.p.m. bicarbonate alkalinity = S

Case 1.—Where S is equal to or less than the total hardness, then S = carbonate hardness

Case 2.—Where S is greater than the total hardness, then the total hardness = the carbonate hardness

Noncarbonate hardness.

P.p.m. total hardness − p.p.m. carbonate hardness = p.p.m. noncarbonate hardness

Magnesium, Volumetric Method.

Reagents: Sec. II (2), (3), (9).
Discussion: Sec. III, page 158.

1. Pipette 100 ml. of the sample into a 250-ml. Erlenmeyer flask.
2. Add just sufficient 0.02N sulfuric acid (3) to neutralize the total alkalinity. (Determine the quantity of acid necessary by titrating a separate 100-ml. portion of the sample, using methyl orange as an indicator.)
3. Boil down to about one-half the volume.
4. Rinse into a 200-ml. volumetric flask, using small portions of freshly boiled, hot, distilled water.
5. Place 100 ml. of freshly boiled, hot, distilled water in a second 200-ml. volumetric flask.
6. Add exactly 25 ml. of a clear solution of saturated limewater (9) to each flask.
7. Fill each to the mark with boiled, hot, distilled water.
8. Stopper loosely with a rubber stopper, and after mixing well set on the steam or water bath for 1 hr.
9. Remove the flasks from the bath and quickly filter a portion of each through filter paper.
10. Immediately pipette a 50-ml. portion of each filtrate into Erlenmeyer flasks. Add 2 drops of phenolphthalein indicator (2) to each flask and titrate with 0.02N sulfuric acid (3) from a burette. Record the ml. of acid used.

Calculations.

(Ml. of 0.02N H_2SO_4 for distilled water − ml. of 0.02N H_2SO_4 for sample) × 9.72 = p.p.m. magnesium as Mg

Sulfate, Volumetric Method.

Reagents: Sec. II (2), (10), (11), (12).
Discussion: Sec. III, page 159.

1. Measure exactly 250 ml. of the sample into a 400-ml. beaker.
2. Add 10 ml. of a 1 per cent solution of hydroxylamine hydrochloride (10). (If the iron content of the sample has previ-

ously been shown to be less than 0.5 p.p.m., this step may be omitted.)

3. Add 20 ml. of benzidine hydrochloride (11).
4. Stir vigorously and allow the precipitate to settle.
5. Filter the solution through paper and wash the beaker and paper thoroughly with small amounts of cold distilled water.
6. Pierce the filter paper in the funnel and wash the precipitate from the paper to the original beaker with about 200 ml. of distilled water. Heat to boiling to dissolve.
7. Add 3 drops of phenolphthalein (2) and titrate with 0.05N sodium hydroxide solution (12) until the first permanent pink color is obtained.
8. Place the filter paper in the solution and continue the titration to the first permanent pink color. Record the ml. of sodium hydroxide used.

Calculations.

Ml. of 0.05N NaOH used $\times$ 9.6 = p.p.m. sulfate (SO_4)

Chlorides.

Reagents: Sec. II (2), (4), (13), (14), (15).
Discussion: Sec. III, page 163.

1. Pipette 50 ml. of the sample into a porcelain evaporating dish.
2. Place about the same quantity of distilled water into a second dish for a color comparison.
3. Add 1 ml. of potassium chromate indicator (13) to each.
4. Add standard silver nitrate solution (14) to the sample from a burette, a few drops at a time, with constant stirring until the first permanent reddish coloration appears. This can be determined by comparison with the distilled-water blank. Record the ml. of silver nitrate used.
5. If more than 7 or 8 ml. of silver nitrate solution are required, the entire procedure should be repeated using a smaller sample diluted to 50 ml. with distilled water.

Calculations.

$$\frac{(\text{Ml. of } AgNO_3 \text{ used} - 0.2) \times 500}{\text{Ml. of sample}} = \text{p.p.m. chloride (Cl)}$$

Precautions.—If the water sample is highly colored, it should be decolorized by shaking with washed aluminum hydroxide (15) and filtering.

If it is highly acid, add 10 per cent sodium carbonate solution until it is slightly alkaline to methyl orange (4).

If it is highly alkaline, add dilute sulfuric acid (63) until it is just acid to phenolphthalein (2).

Iodide.

Reagents: Sec. II (55), (111).

Discussion: Sec. III, page 164.

1. Add about 6 grams of anhydrous sodium carbonate to 50 liters of the water to be tested and evaporate in a large dish or enamel pan until a mushy consistency is obtained. (If a mineral water is to be tested, use from 1 to 10 liters. If a brine is to be tested, use 100 ml.)
2. Cool and add about 100 ml. of 95 per cent alcohol.
3. Heat this mixture and stir thoroughly.
4. Filter through a large filter paper.
5. Treat the solids again as in Step Nos. 2, 3 and 4 and combine the filtrates. Discard the solids.
6. Add a few drops of strong sodium hydroxide (55) to the filtrates and again evaporate to a mushy consistency.
7. Treat these solids as in Step Nos. 2 to 5 above.
8. Add a few drops of sodium hydroxide to the filtrate and evaporate to dryness.
9. Dissolve the solids in 100 ml. of distilled water and rinse into a 250-ml. separatory funnel.
10. Place 5 ml. of the standard potassium iodide solution (111) in a second separatory funnel and add about 100 ml. of distilled water. (See Note below.)
11. Add 5 ml. of concentrated phosphoric acid to each and then exactly 10 ml. of carbon disulfide.
12. Add 20 ml. of hydrogen peroxide to each and mix by rotating for 5 min.
13. Draw off the carbon disulfide, through a pledget of cotton placed in the end of the stem of the funnel, into separate cups of a microcolorimeter.
14. Adjust the colorimeter to the same density of color and read the scale.

Note.—The microcolorimeter scale measures the comparative depths of sample and standard to give the same depth of color. Since the 5 ml. of standard potassium iodide contains 0.1 mg. of iodine, this quantity of iodine

is contained in the carbon disulfide in the standard cup. The iodine content of the sample is calculated from the ratio of the comparative depths as follows:

Calculations.

$$\frac{0.1 \times \text{depth ratio} \times 1{,}000}{\text{Ml. of sample used}} = \text{p.p.m. iodide (I)}$$

Fluoride.

Reagents: Sec. II (112), (113).

Discussion: Sec. III, page 164.

1. Make up a series of color standards by adding 1, 2, 3, etc., up to 10 ml. of standard sodium fluoride solution (112) to a series of 100-ml. Nessler tubes. Make up to the 100-ml. mark with distilled water.
2. Measure 100 ml. of the sample (or a smaller amount diluted to 100 ml. with distilled water) into a 100-ml. Nessler tube.
3. Add 4 ml. of concentrated hydrochloric acid and 1 ml. of zirconium-alizarin solution (113).
4. Mix and allow to stand overnight.
5. Compare the color of the sample with that of the standards and select the standard which matches.

Calculations.

$$\frac{\text{Ml. of standard fluoride in matched standard} \times 10}{\text{Ml. of sample}} = \text{p.p.m. fluoride}$$

Note.—If the total-solids content of the sample is greater than about 500 p.p.m., it will be necessary to dilute the sample and standards with a synthetic water of approximately the same mineral content as the sample. This water is used in place of distilled water for dilution purposes in this test.

Silica, Gravimetric Method.

Discussion: Sec. III, page 165.

1. Place 250 ml. of the well-mixed sample in an evaporating dish. Add a few drops of concentrated hydrochloric acid and evaporate to dryness on the water bath.
2. Moisten the residue with a few drops of concentrated hydrochloric acid.
3. Add about 30 ml. of distilled water and heat to boiling. (More water is sometimes required to dissolve calcium sulfate.)

4. Filter through a quantitative filter paper, rinse the dish and wash the residue on the paper with distilled water, adding the filtered rinsings and washings to the filtrate. All of the insoluble residue in the evaporating dish must be transferred to the filter paper. (The filtrate may be saved for the iron and aluminum or calcium and magnesium determinations.)
5. Ignite and cool a platinum crucible.
6. Fold the paper, place it in the crucible and ignite in the muffle furnace or over a burner (see page 202).
7. Cool in a desiccator and weigh.
8. Add 2 drops of concentrated sulfuric acid and 2 drops of hydrofluoric acid to the residue. Heat gently to volatilize the acids, and ignite. Cool in a desiccator and weigh.

Calculations.

Loss in weight (gm.) $\times$ 4,000 = p.p.m. silica (SiO_2)

Iron and Aluminum Oxides, Gravimetric Method.

Reagents: Sec. II (16), (17).
Discussion: Sec. III, page 159.

1. Place 250 ml. of the well-mixed sample in an evaporating dish. Add a few drops of concentrated hydrochloric acid and evaporate to dryness on a water bath.
2. Moisten the residue with a few drops of concentrated hydrochloric acid.
3. Add about 30 ml. of distilled water and heat to boiling. (More water is sometimes required to dissolve the calcium sulfate.)
4. Filter, rinse the dish and wash the residue on the paper with distilled water, adding the filtered rinsings and washings to the filtrate. (The above steps are omitted if the filtrate from the silica determination is used.)
5. Add a few drops of bromine water (16) to the filtrate and boil for 5 min.
6. Add 10 ml. of 10 per cent solution of ammonium chloride (17), make alkaline to litmus with concentrated ammonium hydroxide and boil again for a few minutes.
7. Filter the precipitated iron and aluminum oxides and rinsings of the container onto a quantitative filter paper and wash the

filter paper with several small quantities of distilled water, adding the washings to the filtrate. (The filtrate may be saved for the determination of calcium and magnesium.)

8. Ignite a crucible, cool in a desiccator and weigh.
9. Fold the filter paper, place it in the crucible and ignite in the furnace or over a burner.
10. Cool in a desiccator and weigh.

Calculations.

Difference in weight (gm.) × 4,000 = p.p.m. combined iron and aluminum oxides (Fe_2O_3 and Al_2O_3)

Note.—To separate the two oxides, make a determination for total iron according to the method given on page 27. The difference between the combined oxides and the calculated iron oxide (Fe_2O_3) is the aluminum oxide (Al_2O_3).

(P.p.m. Fe as determined on page 27) × 1.4298 = p.p.m. Fe_2O_3

Calcium.

Reagents: Sec. II (16), (17), (18), (19), (20).
Discussion: Sec. III, page 160.

1. Evaporate to dryness, in an evaporating dish on a water bath, 250 ml. of the well-mixed sample to which a few drops of concentrated hydrochloric acid have been added.
2. Moisten the residue with a few drops of concentrated hydrochloric acid.
3. Add 30 ml. of distilled water and heat to boiling. (More water is sometimes required to dissolve the calcium sulfate.)
4. Filter, rinse the dish and wash the residue on the paper with distilled water, adding the filtered rinsings and washings to the filtrate. Keep the volume small.
5. Add a few drops of bromine water (16) to the filtrate and boil gently for 5 min.
6. Add 10 ml. of a 10 per cent solution of ammonium chloride (17), make alkaline to litmus with concentrated ammonium hydroxide and boil gently for a few minutes.
7. Filter, rinse the beaker and wash the precipitate on the paper with small portions of distilled water, adding the filtered rinsings and washings to the filtrate. (The above steps are omitted if the filtrate from the iron and aluminum determination is used.)

8. Warm the filtrate and add slowly, with constant stirring, 10 ml. of saturated ammonium oxalate (18).
9. Allow to stand for 30 min. in a warm place.
10. Filter through a quantitative filter paper. Rinse the beaker and wash the precipitate on the paper with small portions of hot distilled water, adding the filtered rinsings and washings to the filtrate. (The filtrate may be saved for the magnesium determination.)
11. Place the original beaker from which the filtration was made under the stem of the funnel, pierce a hole in the filter paper in the funnel and wash the precipitate into the beaker with 30 ml. of 2 per cent sulfuric acid (19).
12. Heat to boiling and add from a burette a standard solution of potassium permanganate (20) until the first permanent pink color is obtained.
13. Transfer the filter paper to the beaker and continue the titration with potassium permanganate to the first permanent pink color. Record the total ml. of permanganate used.

Calculations.

Ml. of $KMnO_4 \times 10$ = p.p.m. calcium (Ca)

Magnesium, Gravimetric Method.

Reagents: Sec. II (16), (17), (18), (21).

Discussion: Sec. III, page 161.

1. Evaporate to dryness, in an evaporating dish over a water bath, 250 ml. of the well-mixed sample to which a few drops of concentrated hydrochloric acid have been added.
2. Moisten the residue with a few drops of concentrated hydrochloric acid.
3. Add 30 ml. of distilled water and heat to boiling. (Sometimes a larger quantity of water is required to dissolve the calcium sulfate.)
4. Filter, rinse the dish and wash the residue on the paper with small portions of water, adding the filtered rinsings and washings to the filtrate.
5. Add a few drops of bromine water (16) and boil for 5 min.
6. Add 10 ml. of 10 per cent solution of ammonium chloride (17), make alkaline to litmus with concentrated ammonium hydroxide and boil gently for a few minutes.

7. Filter, rinse the beaker and wash the residue on the paper with small portions of distilled water, adding the filtered rinsings and washings to the filtrate.
8. Warm the filtrate and add slowly, with constant stirring, 10 ml. of a saturated solution of ammonium oxalate (18).
9. Allow the solution to stand for 30 min. in a warm place.
10. Filter, rinse the beaker and wash the filter paper, adding the filtered rinsings and washings to the filtrate.

(The above steps are omitted, if the filtrate from Step No. 10 of the calcium determination is used.)

11. Add an amount of concentrated ammonium hydroxide to the filtrate equal to about one-ninth its volume and then add 10 ml. of a 10 per cent solution of disodium phosphate (21). Stir the solution vigorously for 5 min.
12. Allow to stand at least 4 hr., and filter the precipitate and several rinsings of the beaker onto a quantitative filter paper. Be sure that all of the precipitate is transferred to the paper.
13. Wash with small portions of 3 per cent ammonia water (110).
14. Ignite, cool and weigh a clean crucible.
15. Transfer the folded filter paper containing the magnesium precipitate to the crucible.
16. Ignite until almost white, cool in a desiccator and weigh.

Calculations.

Gain in weight (gm.) $\times$ 873.6 = p.p.m. magnesium (Mg)

Sulfates, Gravimetric Method.

Reagents: Sec. II (22).

Discussion: Sec. III, page 162.

1. Evaporate to dryness, in an evaporating dish on a water bath, 250 ml. of the sample to which a few drops of concentrated hydrochloric acid have been added.
2. Moisten the residue with a few drops of concentrated hydrochloric acid.
3. Add 30 ml. of distilled water, heat to boiling and filter. (More water is sometimes necessary to dissolve the calcium sulfate.)
4. Rinse the dish and wash the paper with several small portions of distilled water, adding the filtered rinsings and washings to the filtrate.

5. Heat the filtrate to boiling and add 10 ml. of a 10 per cent solution of barium chloride (22) drop by drop with constant stirring.
6. Place the mixture in a warm place for about 30 min.
7. Filter and wash the precipitate on the paper with warm distilled water. Be sure that all of the precipitate is transferred to the paper. (The filtrate may be saved for the sodium and potassium determination.)
8. Ignite, cool and weigh a clean crucible.
9. Fold the filter paper containing the precipitate, place in the crucible, ignite, cool in a desiccator and weigh.

Calculations.

Gain in weight (gm.) $\times$ 1,646.1 = p.p.m. sulfate (SO_4)

Sodium and Potassium, Gravimetric Method.

Reagents: Sec. II (23), (115).

Discussion: Sec. III, page 162.

1. Concentrate 250 ml. of the sample to about 50 ml. by evaporating in a dish on a water bath.
2. Add 10 ml. of a saturated solution of barium hydroxide (23) and heat almost to boiling for 30 min. Keep the dish covered to prevent evaporation. (This precipitates the sulfates which are removed in Step No. 3.)
3. Filter, rinse the dish and wash the residue on filter paper with hot distilled water, adding the filtered rinsings and washings to the filtrate. (The above steps are omitted if the filtrate from sulfate determination is used.)
4. Make the filtrate alkaline with concentrated ammonium hydroxide and add 10 ml. of a fresh 10 per cent solution of ammonium carbonate (115). Heat on the water bath until the precipitate settles, filter and wash. (This removes iron, aluminum, calcium and magnesium.)
5. Evaporate the filtrate to dryness in a dish and ignite at low red heat.
6. Cool the dish and add 10 ml. of hot distilled water, filter and wash, keeping the volume of the filtrate low.
7. Repeat Step Nos. 4, 5 and 6 until no precipitate forms on the addition of the reagents (ammonium hydroxide and ammonium carbonate) to the filtrate from Step No. 6.

8. Transfer the final filtrate and washings to a small platinum dish, add a few drops of concentrated hydrochloric acid and evaporate to dryness.
9. Heat to low red heat, cool and weigh.
10. Add 10 ml. of water to the residue in the dish, filter through quantitative paper and wash the residue. Be sure all of the precipitate is transferred to the paper.
11. Transfer the filter paper to the platinum dish, ignite, cool and weigh.
12. Save the filtrate and washings for the potassium determination, if that test is to be made.

Calculations.

The difference in the weights of the crucible is the weight of the combined sodium and potassium chlorides. This difference in weight × 1,573.6 = p.p.m. sodium and potassium expressed in terms of sodium (Na).

Potassium, Gravimetric Method.

Reagents: Sec. II (24), (25), (63).
Discussion: Sec. III, page 163.

1. Use the filtrate from the sodium and potassium determination or prepare the sample by the procedure given for that test.
2. Add a few drops of 1 to 3 sulfuric acid (63) and 1 ml. of 10 per cent platinic chloride solution (24) for each 30 mg. of combined chlorides.
3. Evaporate almost to dryness on the water bath, remove the dish and allow it to dry at room temperature.
4. To the residue add about 30 ml. of 80 per cent ethyl alcohol (25), stir well so as to dissolve all of the salts soluble in the alcohol.
5. Filter and wash the precipitate with 80 per cent alcohol until the filtrate is colorless.
6. Dry the precipitate on the paper.
7. Dissolve the precipitate by washing it through the paper with small amounts of hot distilled water, catching the filtrate in a platinum dish which has been previously ignited and weighed.
8. Evaporate the solution to dryness, dry for a few minutes at 103°C., cool in a desiccator and weigh.

Calculations.

Difference in weight × 0.3067 = weight of KCl
Weight of combined NaCl and KCl − weight of KCl = weight of NaCl
Weight of KCl (gm.) × 2097.6 = p.p.m. potassium (K)
Weight of NaCl (gm.) × 1573.6 = p.p.m. sodium (Na)

Scheme for Mineral Analysis of Water.

Reagents: Sec. II (2), (3), (4), (13), (14), (16), (17), (18), (19), (20), (21), (22).
Discussion: Sec. III, pages 159 to 163.

Note.—The following scheme has been prepared for the use of those wishing to make what might be termed a "complete mineral analysis" of water. In this scheme the methods given are combined so as to follow step by step in the separation and quantitative determination of each element. The analysis includes the determination of silica, iron and aluminum oxides, calcium, magnesium, sulfate, bicarbonate, chloride and total solids.

Preparation of Sample.

Fill two 250-ml. volumetric flasks to the mark with the sample, add a few drops of concentrated hydrochloric acid and evaporate to dryness in evaporating dishes over a water bath. Cool and moisten each residue with a few drops of concentrated hydrochloric acid. Add about 30 ml. of distilled water and heat to boiling. Filter through separate quantitative filter papers into beakers. Wash the dishes and residues on the papers with small portions of distilled water and add the washings to the filtrates. The volumes of filtrates should be kept below about 60 ml. Save one filtrate for the iron and aluminum oxides and calcium and magnesium determinations and the second for the sulfate determination.

Silica (SiO_2).

Ignite and cool a platinum crucible. Fold one of the filter papers from the above filtration, place it in the crucible and ignite. Cool in a desiccator and weigh. Add 2 drops each of concentrated sulfuric and hydrofluoric acids to the residue. Volatilize the acids. Ignite, cool and weigh the crucible.

Loss in weight (gm.) × 4,000 = p.p.m. silica (SiO_2)
Discard the other filter paper and residue.

First Filtrate.

Iron and Aluminum Oxides.—Add a few ml. of bromine water (16), cover the beaker with a cover glass and boil for a few minutes. Add 20 ml. of 10 per cent ammonium chloride solution (17) and make alkaline to litmus paper with ammonium hydroxide. Cover and boil for 5 min. Ignite, cool and weigh a porcelain crucible. Filter the solution through a quantitative filter paper. Rinse and wash the beaker and filter paper with small portions of distilled water. Save the combined filtrate and washings. Fold the paper, place in the crucible and ignite. Cool and weigh.

Gain in weight (gm.) $\times$ 4,000 = p.p.m. Fe_2O_3 and Al_2O_3

Calcium.—Warm the filtrate from the iron and aluminum determination. Add 10 ml. of a saturated solution of ammonium oxalate (18), drop by drop, with constant stirring and set in a warm place for about 30 min. Filter the solution and wash the beaker and paper with hot distilled water. Save the filtrate and washings for the magnesium determination. Pierce the filter paper and wash the precipitate into the original beaker with about 30 ml. of 2 per cent sulfuric acid (19). (Use a larger quantity of acid if necessary to dissolve the precipitate.) Heat to boiling and titrate with standard potassium permanganate (20). Add the filter paper near the end of the titration.

Ml. of $KMnO_4$ $\times$ 10 = p.p.m. calcium (Ca)

Magnesium.—To the filtrate saved from the calcium determination, add about one-ninth of its volume of concentrated ammonium hydroxide. Add 10 ml. of disodium phosphate solution (21), stir vigorously for 5 min., and allow to stand at least 4 hr. Ignite, cool and weigh a crucible. Filter the precipitate onto quantitative filter paper. Wash the beaker and paper with 3 per cent ammonia solution. Fold the paper and place in the crucible. Ignite, cool and weigh.

Gain in weight (gm.) $\times$ 873.6 = p.p.m. magnesium (Mg)

Second Filtrate.

Sulfate.—Heat to boiling and add, drop by drop with constant stirring, 10 ml. of 10 per cent barium chloride solution (22). Allow the mixture to stand in a warm place with occasional stirring for 30 min. Filter the precipitate onto a quantitative

filter paper and wash with several portions of warm water. Ignite, cool and weigh a crucible. Place the paper and precipitate in the crucible, ignite, cool and weigh.

Gain in weight $\times$ 1,644 = p.p.m. SO_4

Total Solids.—Evaporate 100 ml. of the sample in a weighed evaporating dish. A platinum dish is preferred but others may be used. Dry in the oven at 103°C. for 1 hr., cool in a desiccator and weigh.

Difference in weight (gm.) $\times$ 10,000 = p.p.m. total solids

Chlorides.—Pipette 50 ml. of the sample into an evaporating dish. Add 1 ml. of potassium chromate (13) and titrate with standard silver nitrate (14) to the first permanent reddish coloration.

(Ml. silver nitrate $-$ 0.2) $\times$ 10 = p.p.m. chloride (Cl)

Alkalinity.—Measure 100 ml. of the sample into a flask and add 3 drops of phenolphthalein (2). If a pink color develops, titrate with 0.02N sulfuric acid (3) until the color just disappears. Record the ml. of acid used. Add 3 drops of methyl orange (4) and again titrate with standard acid to the first slight permanent change in color. Record the ml. of acid used. Calculate the alkalinity according to the method given on page 8.

Sodium and Potassium.—These are usually obtained by difference of the positive and negative radicals according to the method given below. If a determination is desired, it may be made on the filtrate from the sulfate determination by following the method for sodium and potassium given on pages 22 to 24.

Calculations.

Discussion: Sec. III, page 165

1. Find the reacting values (R) as follows:

Positive Elements

P.p.m. Ca $\times$ 0.0499 = R of Ca
P.p.m. Mg $\times$ 0.0822 = R of Mg
P.p.m. Fe $\times$ 0.0358 = R of Fe
P.p.m. Al $\times$ 0.1107 = R of Al

Negative Elements

P.p.m. HCO_3 $\times$ 0.0164 = R of HCO_3
P.p.m. CO_3 $\times$ 0.0333 = R of CO_3
P.p.m. SO_4 $\times$ 0.0208 = R of SO_4
P.p.m. Cl $\times$ 0.0282 = R of Cl

Note.—The HCO_3 value used above is obtained by multiplying the bicarbonate alkalinity as $CaCO_3$ by 1.22.

2. (Sum of negative *R* values − sum of positive *R* values) × 23 = p.p.m. sodium and potassium expressed in terms of sodium

Example.

A water analysis showed the following results:

Ca =	131.0 p.p.m.	HCO_3 =	420.0 p.p.m.
Mg =	46.5 p.p.m.	CO_3 =	none
Fe =	0.8 p.p.m.	SO_4 =	111.0 p.p.m.
Al =	1.0 p.p.m.	Cl =	162.5 p.p.m.

To calculate sodium and potassium:

131 × 0.0499 =	6.537	420 × 0.0164 =	6.888
46.5 × 0.0822 =	3.822	0.0 × 0.0333 =	0.0
0.8 × 0.0358 =	0.029	111 × 0.0208 =	2.309
1.0 × 0.1107 =	0.111	162 × 0.0282 =	4.587
	10.499		13.784

(13.784 − 10.499) × 23 = 75.6 p.p.m. Na and K in terms of Na

Total (or Residual) Iron, Colorimetric Method.

Reagents: Sec. II (26), (27), (28), (29), (61).

Discussion: Sec. III, page 166.

1. Evaporate to dryness on a water bath 100 ml. of the sample to which a few drops of concentrated hydrochloric acid have been added. (The residue from the total-solids determination or the final residue from the iron and aluminum oxides determination may be used.)
2. Cool the dish and add about 1 ml. of dilute hydrochloric acid (61).
3. Warm on the water bath. Make sure that the hot acid comes in contact with all of the residue.
4. Add 10 ml. of distilled water and filter into a 100-ml. Nessler tube. Rinse the dish and paper with distilled water.
5. If permanent standards (26) are available, proceed to Step No. 6. If not, prepare temporary standards by measuring 0.1, 0.25, 0.5, 1.0, 1.5, 2.0 and 3.0 ml. of standard iron solution (27) into respective 100-ml. Nessler tubes. Add about 80 ml. of distilled water and 5 ml. of dilute hydrochloric acid to each.
6. Add 2 drops of potassium permanganate (28) to the tube containing the sample (and to each temporary standard if used).

7. Allow to stand for 5 min. and if the color does not persist, add more permanganate.
8. Dilute the sample and standards to the mark.
9. Add 5 ml. of potassium thiocyanate solution (29) to each tube and compare the colors immediately. Record the standard having a color nearest to that of the sample.

Calculations.

a. Using permanent standards

$$\frac{\text{Mg. Fe in permanent standard} \times 1{,}000}{\text{Ml. of sample step 1}} = \text{p.p.m. Fe}$$

b. Using temporary standards

$$\frac{\text{Ml. standard iron} \times 100}{\text{Ml. of sample}} = \text{p.p.m. Fe}$$

Iron, Field Test.

Reagents: Sec. II (26), (27), (28), (29), (61).
Discussion: Sec. III, page 166.

1. Place 100 ml. of the water sample in a Nessler tube or other cylindrical glass tube.
2. Add 5 ml. of dilute hydrochloric acid (61).
3. Add 2 drops of potassium permanganate (28). If pink color does not remain after 5 min., add more permanganate.
4. Add 5 ml. of potassium thiocyanate solution (29).
5. Compare the brown color formed with permanent standards (26) or standard prepared as follows:
   a. Add 100 ml. of distilled water in a Nessler tube.
   b. Add 5 ml. of the dilute hydrochloric acid (61).
   c. Add 5 ml. of potassium thiocyanate solution (29).
   d. Add 0.2 ml. at a time of the standard iron solution (27), until the color of the standard and sample match.

Calculation.

Ml. of the standard iron solution used = p.p.m. of iron (Fe)

Ferrous and Ferric Iron.

Reagents: Sec. II (31), (32), (63).
Discussion: Sec. III, page 166.

1. Prepare color standards by placing in each of eight 100-ml. Nessler tubes 75 ml. of freshly boiled and cooled distilled water, 10 ml. of dilute sulfuric acid (63) and 15 ml. of potassium ferricyanide solution (31).

2. Place in a ninth tube 50 ml. of the sample, 10 ml. of dilute sulfuric acid (63), 15 ml. of potassium ferricyanide solution (31) and dilute to the mark with freshly boiled and cooled distilled water.
3. Add to the eight standard tubes 0.5, 1.0, 1.5, 2.0, 2.5, 3.0, 3.5 and 4.0 ml., respectively, of standard ferrous ammonium sulfate iron solution (32).
4. Mix and compare the blue color which develops in the sample with that of the standards. Record the standard having a color nearest to that of the sample. (Ferrous iron is not present if no color develops in the sample.)

Calculations.

$$\frac{\text{Ml. standard iron solution} \times 100}{\text{Ml. of sample}} = \text{p.p.m. ferrous iron}$$

P.p.m. total iron — p.p.m. ferrous iron = p.p.m. ferric iron

Manganese, Colorimetric Method.

Reagents: Sec. II (33), (34), (35).

Discussion: Sec. III, page 167.

1. Make up standards by placing 0.2, 0.4, 0.6, 0.8, 1.0 and 1.5 ml. of standard manganous sulfate solution (33) into 250-ml. Erlenmeyer flasks and adding 50 ml. of distilled water.
2. Measure 100 ml. of the sample into a similar flask.
3. Add 2 ml. of 1 to 1 nitric acid (34) to the sample and to each standard.
4. Heat the sample and standards to boiling.
5. Determine the volume of silver nitrate solution (35) necessary to precipitate the chlorides by titrating a separate portion of the sample as described in the chloride determination on pages 15 and 16.
6. Add 1 ml. of silver nitrate solution (35) to each standard. Add 1 ml. more than the volume of silver nitrate as determined in Step No. 5 to the sample.
7. Shake and heat the flask containing the sample.
8. Filter the sample and rinsings into an Erlenmeyer flask. Wash the filter paper with distilled water.
9. Add about 0.5 gram of ammonium persulfate crystals to each flask (standards and sample), mix and warm for 10 min.

10. Transfer to 100-ml. Nessler tubes, make up to the mark with distilled water and compare the colors. Record the standard having the color nearest that of the sample.

Calculations.

$$\frac{\text{Ml. standard manganous solution} \times 100}{\text{Ml. of sample}} = \text{p.p.m. Mn}$$

Residual Alum (Aluminum), Colorimetric Method.

Reagents: Sec. II (36), (37), (38), (39), (40).
Discussion: Sec. III, page 167.

1. Prepare standards in 200-ml. phosphoric acid flasks (Erlenmeyer flasks may be used) by diluting 1, 2 and 3, up to 8 ml. of the standard alum solution (36) to 50 ml. with distilled water. Permanent standards (37) may be used.
2. Place 50 ml. of the sample into a similar flask.
3. To each flask add 1 ml. of alizarin red S solution (38) and boil for 2 min.
4. Add sodium bicarbonate solution (39), drop by drop, until a slight purple tinge is obtained.
5. Add exactly 0.5 ml. excess of the bicarbonate solution, boil for 2 min. Cool, rinse into Nessler tubes and make up to 50 ml. with distilled water.
6. Add 1 ml. of acetic acid solution (40), let stand 1 min. and compare the colors. Record the standard nearest to the color of the sample.

Calculations.

$$\frac{\text{Ml. standard alum} \times 10}{\text{Ml. of sample}} = \text{p.p.m. alum as } Al_2O_3$$

Residual Chlorine, Orthotolidine Method.

Reagents: Sec. II (4), (30), (66), (67), (95).
Discussion: Sec. III, page 169.

1. Place 100 ml. of the sample in a 100-ml. Nessler tube. If the temperature of the sample is below 20°C., bring it up to this temperature by immersing the tube in a bath of warm water.
2. Add 1 ml. of orthotolidine reagent (66) and mix.
3. Allow the tube to stand in a dark place for 5 min.
4. Compare the color which develops with that of permanent standards (67) by looking down through the tubes against

a white background illuminated with good light. The chlorine value, in p.p.m., of the standard most nearly matching the color of the sample equals the p.p.m. chlorine of the sample.

Note.—Free chlorine can be distinguished from chloramines owing to its decolorization of methyl orange in acid solution, as follows:

1. Determine alkalinity of the water using methyl orange indicator (4).
2. To another 100-ml. portion of the water add sufficient acid to overcome the alkalinity and then add 2 ml. of 0.1N sulfuric acid (95) in excess.
3. Add 0.2-ml. increments of dilute methyl orange (30) until the pink color becomes permanent.

Calculations.

Ml. of dilute methyl orange × 0.1 = p.p.m. free chlorine

Residual Chlorine, Starch-iodide Method.

Reagents: Sec. II (60), (68).

Discussion: Sec. III, page 169.

1. Place 200 ml. of the sample in an Erlenmeyer flask. Chill to 20°C. or lower by immersing the flask in a bath of ice water.
2. Add a small crystal of potassium iodide and 1 ml. of concentrated hydrochloric acid.
3. Add 1 ml. of starch solution (60). A blue color shows the presence of chlorine.
4. For a quantitative estimation, titrate the solution with 0.001N sodium thiosulfate solution (68) until the blue color just disappears. Record the ml. of thiosulfate used.

Calculations.

Ml. of 0.001N thiosulfate × 0.1773 = p.p.m. residual chlorine

Break-point Chlorination.

Reagents: Sec. II (66), (66A), (67), (75).

Discussion: Sec. III, pages 169 and 170.

1. Place 200-ml. portions of the water (not chlorinated) to be tested in each of ten 8-oz. bottles.
2. Add 0.1 ml. of chlorine water (75) to the first bottle, 0.2 ml. to the second, and so on, increasing the amounts by 0.1 ml. until all ten bottles have been treated. (As chlorine break point occurs over a very wide range in different waters, it may be necessary to use a larger number of bottles in the series or to use smaller or larger doses of chlorine water.)

Chlorine used (p.p.m.) = 5 × ml. of chlorine water used.

3. Shake each bottle gently and allow to stand 30 min.
4. Run residual chlorine on each bottle by method given on page 30.
5. Plot the results on coordinate paper with chlorine residuals as ordinates and p.p.m. of chlorine used as abscissas. The point at which the residual-chlorine value starts to decrease is the break point.

Note.—Flash test to determine whether or not break-point chlorination has been exceeded (as developed by Paul C. Laux and J. B. Nickel) may be made as follows:

1. Place 100 ml. of the chlorinated water in a 250-ml. Erlenmeyer flask.
2. Add 1 ml. of orthotolidine reagent (66A) and mix by swirling the flask.
3. Immediate appearance of blue or yellow color indicates that the chlorine break point has been exceeded. If no color appears, the residual chlorine is just at or is below the break point.

Color.

Reagents: Sec. II (41).

1. Fill a 100-ml. Nessler tube to the mark with the water to be tested. If the turbidity is high, it should be removed by centrifuging or settling (not by filtering).
2. Compare this color with that of standards (41) by looking vertically down through the tubes at a white surface in good light.
3. If the color is greater than that of the standards, the water should be diluted with distilled water until within the range.

Calculations.

$$\frac{\text{Ml. of standard platinum solution} \times 500}{\text{Ml. of sample}} = \text{color}$$

Turbidity.

Reagents: Sec. II (42), (43).

For turbidities above 25 use the Jackson turbidimeter as follows:

1. See that the candle is at its full height in the holder. Remove such portion of the charred part of the string as can be easily broken off with the fingers.
2. Light the candle and immediately start Step No. 3. (Do not allow the tube to become hot before pouring water into it.)

3. Pour the water to be tested into the tube until the image of the candle flame just diappears from view. The water should be poured very slowly after the image becomes only faintly visible. Greater accuracy may be secured by making the observation in a darkened room or with a black cloth over the head.
4. Read the turbidity from the scale on the graduated tube.

For turbidities from 5 to 25:

1. Place the sample in a 1-liter bottle of clear glass similar to that used for the standards (42).
2. Shake the sample and each standard vigorously and compare the turbidities by looking horizontally through the bottles at some object. Note the distinctness with which the outlines of the object can be seen.
3. Record the turbidity of the standard most nearly matching the sample.

For turbidities less than 5 use the Baylis turbidimeter as follows:

1. Fill the tubes with the sample and allow to stand until the air bubbles rise to the surface.
2. Select the standard (43) which seems to have a turbidity nearest that of the sample and place it and the tube containing the sample in the turbidimeter.
3. Compare the sample with the various standards until that having the same turbidity as the sample has been determined. (Equal turbidities in the two tubes will give equal intensities of blue light.)
4. If the sample has a turbidity slightly above 5, it should be diluted until it comes within the range of this instrument. The turbidity is then calculated by multiplying the turbidity of the diluted sample by the dilution ratio.

Note.—Directions for making the Baylis turbidimeter are given in the *Journal of Industrial and Engineering Chemistry*, vol. 18, page 311, 1926.

Odor, Qualitative Method.

Cold odor:

1. Fill an Erlenmeyer flask about half-full of the sample and cover with a watch glass.

2. Shake vigorously, remove the cover and smell the odor at the mouth of the flask.
3. Describe the odor as indicated below.

Hot odor:

1. Place 150 ml. of the sample in a 500-ml. Erlenmeyer flask.
2. Cover with a watch glass and heat to approximately 65°C.
3. Shake vigorously, remove the cover and smell the odor.
4. Describe the odor as indicated below.

The results are expressed by using combinations of the numbers and letters given below:

0—no odor	*e*—earthy
1—very faint	*f*—fishy
2—faint	*g*—grassy
3—distinct	*m*—moldy
4—decided	*M*—musty
5—very strong	*P*—peaty
a—aromatic	*s*—sweetish
c—free chlorine	*S*—sulfide
d—disagreeable	*v*—vegetable

Odor, Quantitative Estimation.

1. Boil a quantity of distilled water until free from odor, or pass the water through a filter of activated carbon until odor-free. Cool to room temperature.
2. Clean six or more 500-ml. Erlenmeyer flasks and remove the last traces of odor by boiling water in them for 5 min.
3. Discard the water, cool the flasks and cover them with watch glasses.
4. Determine the approximate odor as follows:
   a. Place 100 ml. of odor-free water, prepared in Step No. 1, in each of two flasks.
   b. Warm to 60°C. and add to one flask 5 ml. of the sample.
   c. Shake both flasks vigorously. Remove the cover and determine if the water in the flask receiving the sample has an odor when compared with that in the other flask.
   d. If the odor is present, repeat using a smaller sample.
   e. If not present, add more of the sample to the water in the flask until the odor is present.
5. Knowing the approximate odor, a more accurate determination may be made as follows:

a. Add 100 ml. of the odor-free water to each of the several flasks.
b. Cover with watch glasses and warm to 60°C.
c. To the first five flasks add increasing volumes of the sample, including the volume determined by the approximate method. For example, if the odor was found using 5 ml. of the sample in the approximate method, add 3, 4, 5, 6 and 7 ml., respectively, to the five flasks.
d. Shake vigorously and determine the flask in which the odor first appears.

Note.—If more than 5 ml. is required, reduce the volume of odor-free water used so that the total volume is not over 105 ml.

Calculations.

$$\frac{\text{Ml. odor-free water} + \text{ml. of sample}}{\text{Ml. of sample}} = \text{odor value}$$

Ammonia Nitrogen.

Reagents: Sec. II (44), (45), (46), (47), (49).
Discussion: Sec. III, page 171.

1. Clear the distillation apparatus of ammonia by boiling distilled water in it. Use a liter Kjeldahl flask.
2. Empty the flask and measure 500 ml. of the sample into it. If a smaller sample is used, make up to 500 ml. with ammonia-free water (44).
3. Add 10 ml. of phosphate buffer solution (49) and distill until almost 200 ml. are collected in a 200-ml. volumetric flask. (The residue may be used for either the albuminoid or organic nitrogen determination.)
4. Make up the distillate to 200 ml. with ammonia-free water and mix well.
5. Pipette a measured portion into a 100-ml. Nessler tube and make up to the mark with ammonia-free water. The volume required must be determined by trial. If the color produced by this volume in Step No. 7 does not come within the range of the standards, use a volume which will come within the range.
6. If permanent ammonia standards (45) are available, proceed to Step No. 7. If not, make up temporary standards by adding 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.7, 2.0, 2.5 and 3.0 ml. of

standard ammonium chloride solution (46) to 100-ml. Nessler tubes. Dilute to the mark with ammonia-free water.

7. Add 2 ml. of Nessler reagent (47) to the sample (and to each temporary standard, if used), allow to stand 10 min. and compare the colors. Record the standard having the color nearest that of the sample.

Calculations.

a. Using permanent standards

$$\frac{\text{Mg. N in permanent standard} \times 200{,}000}{\text{Ml. of sample Step 2} \times \text{ml. of portion Step 5}} = \text{p.p.m. ammonia nitrogen as N}$$

b. Using temporary standards

$$\frac{\text{Ml. of standard } NH_4Cl \times 2{,}000}{\text{Ml. used in Step 5} \times \text{ml. of sample}} = \text{p.p.m. ammonia nitrogen as N}$$

P.p.m. ammonia as N $\times$ 1.216 = p.p.m. ammonia as NH_3

Albuminoid Nitrogen.

See page 63.

Organic Nitrogen.

See page 64.

Nitrite Nitrogen.

Reagents: Sec. II (50), (51), (52).

Discussion: Sec. III, page 172.

1. Place 100 ml. of the sample into a 100-ml. Nessler tube. (If the nitrite content proves to be high, a smaller volume diluted to 100 ml. with distilled water should be used.)
2. Prepare standards by adding 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.7, 2.0 and 2.5 ml. of standard sodium nitrite solution (50) to 100-ml. Nessler tubes and dilute to the mark with distilled water.
3. Add 2 ml. of sulfanilic acid solution (51) and 2 ml. of a-naphthylamine acetate solution (52) to the sample and to each standard.
4. Mix and after 10 min. compare the colors and select the standard having a color nearest to that of the sample

Calculations.

$$\frac{\text{Ml. standard } NaNO_2 \times 0.5}{\text{Ml. of sample}} = \text{p.p.m. nitrite nitrogen as N}$$

P.p.m. nitrite nitrogen as N $\times$ 3.284 = p.p.m. nitrite as NO_2

Nitrate Nitrogen, Phenoldisulfonic Acid Method.

Reagents: Sec. II (3), (15), (53), (54), (55), (56).

Discussion: Sec. III, page 172.

1. Determine the number of ml. of 0.02N sulfuric acid (3) necessary to neutralize the methyl orange alkalinity of 100 ml. of the sample. See page 8. Also, determine the number of ml. of silver sulfate solution (53) necessary to precipitate the chlorides in 100 ml. of sample. See page 15.
2. If the sample is highly colored, stir a 200-ml. portion with a small amount of aluminum hydroxide (15) and filter.
3. Pipette 100 ml. of the sample or the clarified filtrate into an evaporating dish and add the quantity of 0.02N sulfuric acid as determined in Step No. 1.
4. If less than 3 ml. of the silver sulfate solution were required in Step No. 1, omit Step No. 4. If more than 3 ml. were required, add slightly less than the volume required to the sample in the evaporating dish. Add a small amount of aluminum hydroxide (15), mix and filter into a second dish.
5. Evaporate the sample from Step No. 3 or the filtrate from Step No. 4 to dryness on the water bath.
6. Add 3 ml. of phenoldisulfonic acid (54) and rub in well with a glass rod to insure contact of the acid with the residue.
7. Dilute with 20 ml. of distilled water and add slowly, with stirring, a solution of sodium hydroxide (55) until the maximum yellow color is developed. Not more than 6 ml. will be required. If no color develops, nitrate is absent.
8. Filter into a 100-ml. Nessler tube and rinse the dish and paper with distilled water, adding the rinsings to the tube until all of the colored solution has been transferred. Dilute to the mark with distilled water.
9. Prepare standards by adding 0.2, 0.5, 0.75, 1.0, 3.0, 5.0, 10, 20, 30 and 40 ml. of the standard nitrate solution (56) to 100-ml. Nessler tubes. Dilute to the mark and add 2 ml. of sodium hydroxide (55) to each tube.
10. Compare the colors and select the standard having a color nearest to that of the sample.

Calculations.

$$\frac{\text{Ml. of standard nitrate} \times 10}{\text{Ml. of sample}} = \text{p.p.m. nitrate nitrogen as N}$$

$$\text{P.p.m. nitrate as N} \times 4.427 = \text{p.p.m. nitrate as NO}_3$$

Dissolved Oxygen, Winkler Method.

Reagents: Sec. II (57), (58), (59), (60).
Discussion: Sec. III, page 174.

Note.—This method may be used for the majority of dissolved-oxygen determinations made in the water laboratory because it has been modified by the addition of sodium azide to the alkaline potassium iodide solution to eliminate nitrite interference. In cases where a water high in iron or organic matter is encountered, the Rideal-Stewart modification given on page 39 should be used.

1. Collect the sample in an 8-oz. glass-stoppered bottle. Be very careful to avoid contact of the sample with air. The bottle should be completely filled. (See Sampling for Dissolved Oxygen, page 196.)
2. Immediately after collection, add 1 ml. of manganous sulfate solution (57) by means of pipette, dipping the end of the pipette just below the surface of the water.
3. Add 1 ml. of alkaline potassium iodide solution (58) in a similar manner.
4. Insert the stopper and mix by inverting the bottle several times.
5. Allow the precipitate to settle halfway and mix again.
6. Again allow the precipitate to settle halfway.
7. Add 1 ml. of concentrated sulfuric acid. Insert the stopper at once after the addition of the acid and mix as before.
8. Allow the solution to stand at least 5 min.
9. Withdraw 100 ml. of the solution into an Erlenmeyer flask and immediately add 0.025N sodium thiosulfate (59), drop by drop, from a burette until the yellow color almost disappears.
10. Add about 1 ml. of starch solution (60) and continue the addition of the thiosulfate until the blue color just disappears. Record the ml. of thiosulfate used. (Disregard any return of the blue color.)

Calculations.

Ml. of 0.025N sodium thiosulfate $\times$ 2 = p.p.m. dissolved oxygen
(See page 216 for Dissolved Oxygen Saturation Table.)

Dissolved Oxygen, Rideal-Stewart Modification.

Reagents: Sec. II (28), (57), (58), (59), (60), (62).
Discussion: Sec. III, page 176.

1. Collect the sample in an 8-oz. glass-stoppered bottle, being careful to avoid contact of the sample with the air. The bottle should be completely filled. (See Sampling for Dissolved Oxygen, page 196.)
2. Immediately after collecting, add 0.7 ml. of concentrated sulfuric acid and 1 ml. of potassium permanganate (28) by means of pipettes, dipping the ends just below the surface of the water.
3. Insert the stopper and mix by inverting the bottle several times.
4. If the color of the permanganate does not last for 20 min., add 1 ml. more. If this is not sufficient, use a stronger solution.
5. After 20 min., add 1 ml. of potassium oxalate (62), insert the the stopper and mix.
6. After the color has disappeared, add 1 ml. of manganous sulfate (57) and 3 ml. of alkaline potassium iodide (58), insert the stopper and mix.
7. Allow the precipitate to settle halfway and mix again.
8. Again allow the precipitate to settle halfway.
9. Add 1 ml. of concentrated sulfuric acid and immediately insert the stopper and mix.
10. Allow the solution to stand at least 5 min. (The solution may stand several hours in this condition without harm.)
11. Withdraw 100 ml. of the solution into an Erlenmeyer flask and add 0.025N sodium thiosulfate (59), drop by drop, from a burette until the yellow color almost disappears.
12. Add 1 ml. of starch solution (60) and continue the addition of the thiosulfate until the blue color just disappears. Record the ml. of thiosulfate used. (Disregard any return of the blue color.)

Calculations.

Ml. of 0.025N sodium thiosulfate $\times$ 2 = p.p.m. dissolved oxygen
(See page 216 for Dissolved Oxygen Table.)

Oxygen Consumed.

Reagents: Sec. II (63), (64), (65).
Discussion: Sec. III, page 173.

1. Pipette 100 ml. of the sample into a 250-ml. Erlenmeyer flask.
2. Add 10 ml. of dilute sulfuric acid (63) and 10 ml. of standard potassium permanganate (65).
3. Heat in a boiling-water bath for exactly 30 min., keeping the water in the bath above the level of the solution in the flask.
4. If the solution becomes faintly colored, repeat the above using a smaller sample diluted to 100 ml. with distilled water.
5. After 30 min. in the water bath, add 10 ml. of standard ammonium oxalate (64).
6. Titrate while hot with standard potassium permanganate to the first pink coloration. Record the ml. of permanganate used.

Calculations.

$$\frac{\text{Ml. of potassium permanganate used in Step No. 6} \times 100}{\text{Ml. of sample}} = \text{p.p.m. oxygen consumed}$$

Salt Analysis.

Reagents: Sec. II (4), (13), (14), (20), (21), (22), (63), (105).
Discussion: Sec. III, pages 160 to 162.

A. Moisture.

1. Dry a clean evaporating dish in the oven at 103°C. for 2 hr. Cool in a desiccator and weigh.
2. Add exactly 20 grams of the salt to the evaporating dish and reweigh. (Other amounts of salt may be used but 20 grams is generally satisfactory to obtain accurate results.)
3. Dry in an oven at 103°C. for 2 to 3 hr., cool in desiccator and reweigh. (Oven temperature must be kept well below 120°C. or water of crystallization may be lost.)

Calculations.

$$\frac{\text{Loss in weight (gm.)} \times 100}{20} = \text{per cent moisture}$$

Per cent solids = 100 − per cent moisture

B. Calcium.

1. Transfer the salt from *A* (or start with a new 20-gram weighed sample) to a 250-ml. beaker and add about 250 ml. of distilled water and 5 ml. of concentrated hydrochloric acid.
2. Heat to boiling and add 25 ml. of calcium reagent (105) and 3 drops of methyl orange indicator (4).
3. Heat to boiling and slowly add concentrated ammonium hydroxide until distinctly alkaline to litmus.
4. Boil gently for a few minutes and allow to settle on a steam bath until clear.
5. Filter, first by decantation, then rinse the beaker and wash the precipitate on the filter paper with hot distilled water until free of chlorides. (Save this filtrate for magnesium determination.)
6. Place the original beaker from which the filtration was made under the stem of the funnel, pierce a hole in the filter paper, wash the precipitate through the hole with hot distilled water and finally wash the paper with 20 ml. of 1 to 3 sulfuric acid (63). The total volume should be about 150 ml.
7. Heat nearly to boiling and titrate while hot with 0.125N potassium permanganate (20) until permanent pink color is obtained.
8. Transfer the filter paper to the beaker and continue the titration with the potassium permanganate until a permanent pink color is obtained. Record the ml. of permanganate used.

Calculations.

Total ml. of $KMnO_4 \times 0.0025$ = gm. of calcium in sample

C. Magnesium.

1. Use the filtrate from Step No. 5 of the calcium determination. Add 50 ml. of 10 per cent solution of disodium phosphate (21).
2. Add 20 ml. of concentrated ammonium hydroxide slowly, stirring vigorously.
3. Allow to stand at least 4 hr. (overnight preferred).
4. Filter the precipitate and several rinsings of the container onto a quantitative filter paper. Be sure all the precipitate is transferred to the paper.

5. Wash the precipitate on the filter free from chlorides with small portions of dilute ammonia water (40 ml. concentrated NH_4OH to 400 ml. of distilled water).
6. Ignite, cool and weigh a clean porcelain crucible.
7. Transfer the folded filter paper containing the magnesium precipitate ($MgNH_4PO_4$) to the crucible and dry in an oven at 103°C.
8. Ignite the crucible and contents in a muffle furnace or over a Meeker burner until residue is almost white. Cool in a desiccator and weigh as $Mg_2P_2O_7$.

Calculations.

Gain in weight (gm.) = gm. of $Mg_2P_2O_7$
Gain in weight (gm.) $\times$ 0.8553 = gm. of Mg as $MgCl_2$
Gain in weight (gm.) $\times$ 0.1091 = gm. of Mg

D. Sulfates.

1. Weigh exactly 20 grams of the salt, transfer to a 250-ml. beaker, add about 150 ml. of distilled water and 5 ml. of concentrated hydrochloric acid.
2. Heat to boiling, and while gently boiling add 10 ml. of 10 per cent barium chloride (22) drop by drop, with constant stirring, and continue boiling for 2 min.
3. Let settle in a warm place for 30 min. or more.
4. Filter and wash the filter with hot water. (Be sure that all the precipitate is transferred from the beaker to the paper.)
5. Ignite, cool and weigh a clean crucible.
6. Transfer the folded filter paper containing the precipitate ($BaSO_4$) to the crucible, ignite, cool in a desiccator and weigh as $BaSO_4$.

Calculations.

Gain in weight (gm.) = gm. of $BaSO_4$
Gain in weight (gm.) $\times$ 0.4115 = gm. SO_4

E. Chloride.

1. Dissolve exactly 1 gram of the salt in a small quantity of distilled water and make up to 1 liter.
2. Pipette 10 ml. of this solution into a porcelain evaporating dish and add distilled water to make the volume about 50 ml.

3. Add 1 ml. of potassium chromate indicator (13).
4. Add standard silver nitrate solution (14) from a burette with constant stirring until the first permanent reddish color is obtained.

Calculations.

(Ml. of $AgNO_3$ solution used − 0.2) × 0.05 = total gm. of chloride (Cl) in 1-gram sample

F. Final Calculations.

1. *a.* If the calcium is in excess of the sulfates, calculate all sulfates to $CaSO_4$ and excess calcium to $CaCl_2$.
   b. If the sulfates are in excess of the calcium, calculate all calcium to $CaSO_4$ and excess sulfate to Na_2SO_4.
2. Calculate sum of chlorides (Cl) in the $CaCl_2$ and $MgCl_2$.
3. Subtract this value from total chlorides in 20-gram sample and then multiply by factor to change Cl to NaCl.

Illustrative Example 1. (Condition 1*a*, above.)

Analysis of 20-gm. sample gave

Moisture	0.1000 gm.
Calcium (Ca)	0.0955 gm.
$MgCl_2$	0.3890 gm.
$BaSO_4$	0.4422 gm.
Chlorides (Cl)	12.00 gm.

Solution.

$$\text{Barium sulfate as calcium} = 0.4422 \times \frac{40.08}{233.42} = 0.0760 \text{ gm.}$$

$$\text{Excess calcium as } CaCl_2 = (0.0955 - 0.0769) \times \frac{110.99}{40.08} = 0.0540 \text{ gm.}$$

$$\text{Chlorides in } CaCl_2 \text{ and } MgCl_2 = \frac{70.92}{110.99} \times 0.0540 + \frac{70.92}{95.23} \times 0.3890 = 0.3241 \text{ gm.}$$

$$\text{Chlorides as sodium chloride} = (12.00 - 0.3241) \times \frac{58.45}{35.457} = 19.24 \text{ gm.}$$

$$\text{Per cent sodium chloride in sample} = \frac{19.24}{20} \times 100 = 96.2$$

Note.—The NaCl may also be calculated by the method given on page 27, but instead of p.p.m. use grams in 20 grams.

Illustrative Example 2. (Condition 1*b*, above.)

Analysis same as in Example 1 except calcium 0.0455 gm.

Solution.

$$\text{Calcium as } BaSO_4 = 0.0455 \times \frac{233.42}{40.08} = 0.2603 \text{ gm.}$$

$$\text{Chloride in } MgCl_2 = 0.3890 \times \frac{70.92}{95.23} = 0.2895 \text{ gm.}$$

$$\text{Chloride as sodium chloride } (12.00 - 0.2895) \times \frac{58.45}{35.46} = 19.30 \text{ gm.}$$

$$\text{Per cent sodium chloride in sample} = \frac{19.30}{20} \times 100 = 96.5$$

Problems for Solution.

1. Analysis of a 20-gm. salt sample gave moisture 0.0550 gm., calcium 0.0755 gm., magnesium chloride 0.1890 gm., barium sulfate 0.3422 gm and chloride (Cl) 12.0076 gm. Calculate per cent NaCl (*a*) by method given in above illustrative problems and (*b*) by method on page 27?

Ans. 97.7 per cent.

2. Same problem as 1 except calcium 0.0400 gm., magnesium chloride 0.089 gm. and chloride (Cl) 12.1076 gm. *Ans.* 99.3 per cent.

Lime, Percentage of Oxide or Hydroxide.

Reagents: Sec. II (2), (4), (95).

Discussion: Sec. III, page 190.

1. Pulverize the sample and sift through a screen containing 50 openings per linear inch.
2. Weigh 0.5 gram of the sample into a liter glass-stoppered bottle.
3. Add 500 ml. of freshly boiled and cooled distilled water.
4. Shake occasionally for a period of about 1 hr. and allow to settle overnight.
5. Pipette 50 ml. of the clear supernatant liquor into a 250-ml. Erlenmeyer flask, add 3 drops of phenolphthalein (2) and titrate with 0.1N sulfuric acid (95) until the pink color just disappears. Record the ml. of acid used.
6. Add 3 drops of methyl orange (4) and again titrate with the 0.1N acid to the first change in color. Record the ml. of acid used.

Calculations.

Let T = total ml. of acid used for both the phenolphthalein and methyl orange titrations

Let P = ml. of acid used for the phenolphthalein titration
If the sample was hydrated lime:

$$(2P - T) \times 7.4 = \text{per cent hydroxide as } Ca(OH)_2$$

If the sample was quicklime:

$$(2P - T) \times 5.6 = \text{per cent oxide as } CaO$$

Note.—These values represent content of both calcium and magnesium hydroxides and oxides. If calcium only is desired, use method given below.

Lime, Percentage of Calcium.

Reagents: Sec. II (17), (18).
Discussion: Sec. III, page 190.

1. Dry a portion of the lime sample in the 103°C. oven for 1 hr Cool in a desiccator and transfer to a dry weighing bottle.
2. Weigh the bottle and contents. Pour about 0.3 gram of the lime into a beaker and again weigh the bottle and contents. The difference in weight is the weight of the dry lime sample used.
3. Add about 30 ml. of 1 to 1 hydrochloric acid and heat gently for about 5 min.
4. Filter and rinse the beaker and paper with small portions of hot distilled water. Add the filtered rinsings to the filtrate. (The paper may be ignited in a weighed crucible and the ash reported as insoluble matter.)
5. Add 10 ml. of 10 per cent ammonium chloride solution (17) to the filtrate and make alkaline to litmus with concentrated ammonium hydroxide.
6. If a precipitate of iron and aluminum oxides forms, filter and wash the beaker and paper with distilled water. Add the filtered washings to the filtrate.
7. Heat to boiling and add slowly, with stirring, 20 ml. of a saturated solution of ammonium oxalate (18).
8. Allow to stand in a warm place for 30 min.
9. Ignite, cool and weigh a crucible.
10. Filter the precipitate onto a quantitative filter paper and wash well with small portions of distilled water.
11. Test the filtrate with a few drops of ammonium oxalate. If a precipitate is obtained, repeat Step Nos. 7, 8, 10 and 11. In repeating Step No. 10, filter through the filter paper previously used.

12. Place the paper and precipitate in the weighed crucible and ignite to bright red heat for 30 min., cool in the desiccator and weigh. Repeat the ignition until no further loss in weight is obtained.

Calculations.

If hydrated lime is used:

$$\frac{\text{Gain in weight (gm.)} \times 132}{\text{Weight of sample (gm.)}} = \text{per cent calcium as } Ca(OH)_2$$

If quicklime is used:

$$\frac{\text{Gain in weight (gm.)} \times 100}{\text{Weight of sample (gm.)}} = \text{per cent calcium as CaO}$$

Soda Ash, Percentage of Sodium Carbonate.

Reagents: Sec. II (4), (95).

Discussion: Sec. III, page 192.

1. Dissolve 0.53 gram of the sample in boiled and cooled distilled water in a 250-ml. volumetric flask.
2. Make up to the mark with boiled and cooled distilled water and mix.
3. Pipette 50 ml. of the solution into an Erlenmeyer flask and add 3 drops of methyl orange (4).
4. Titrate with 0.1N sulfuric acid (95) to the first slight permanent change in color. Record the ml. of acid used.

Calculations.

Ml. of 0.1N acid $\times$ 5 = per cent Na_2CO_3

Alum, Percentage of Aluminum Oxide.

Reagents: Sec. II (17).

Discussion: Sec. III, pages 159, 160 and 191.

1. Weigh about 0.5 gram of the alum into a beaker and dissolve in about 100 ml. of distilled water.
2. Filter off any insoluble residue, rinse the beaker and paper with distilled water and add the filtered rinsings to the filtrate.
3. Add 10 ml. of 10 per cent ammonium chloride (17) and make alkaline to litmus with concentrated ammonium hydroxide.
4. Cover the beaker with a cover glass and boil gently for about 10 min.

5. Filter onto a quantitative filter paper and wash the beaker and paper several times with hot distilled water.
6. Ignite, cool and weigh a crucible.
7. Fold the paper, place it in the weighed crucible, dry and ignite at a bright red heat for at least 10 min.
8. Cool in a desiccator and weigh.

Calculations.

$$\frac{\text{Gain in weight (gm.)} \times 100}{\text{Weight of sample (gm.)}} = \text{per cent } Al_2O_3$$

Per cent $Al_2O_3 \times 3.35$ = per cent $Al_2(SO_4)_3$

Per cent $Al_2O_3 \times 6.537$ = per cent $Al_2(SO_4)_3.18H_2O$. (This value may be greater than 100 per cent if the sample contains less than 18 molecules of water. See page 147.)

Iron in Coagulants.

For method see "Iron, Volumetric Method," page 84.

Chlorine in Hypochlorites.

Reagents: Sec. II (59), (60).

Discussion: Sec. III, page 191.

1. Fill a clean, dry weighing bottle about half-full with the powder. Insert the stopper and weigh.
2. Place about 100 ml. of water in a beaker.
3. Carefully remove the stopper from the weighing bottle and pour about 0.7 gram of the powder into the water in the beaker.
4. Insert the stopper and reweigh. The difference in weight is the weight of the sample used.
5. Thoroughly mix the powder with the water and pour the solution and several rinsings of the beaker into a 200-ml. volumetric flask.
6. Fill to the mark with distilled water. Mix thoroughly.
7. Dissolve about 2 grams of potassium iodide and 2 ml. of glacial acetic acid in 50 ml. of distilled water in an Erlenmeyer flask.
8. Pipette exactly 25 ml. of the chlorine solution into the flask.
9. Titrate with 0.025N sodium thiosulfate (59), using starch as an indicator (60) near the end of the titration.

Calculations.

Chlorine in sample:

$$\frac{\text{Ml. 0.025N thiosulfate} \times 0.7091}{\text{Gm. of sample used}} = \text{per cent}$$

Activated Carbon Evaluation.

Reagents: Sec. II (63), (103), (104), (106), (107), (108), (109).

Note.—The following is the Fox and Gauge method as modified by Kershaw of the Indianapolis Water Co.

1. Pipette 100-ml. portions of standard phenol solution (109) into each of five liter volumetric flasks and dilute to the mark with distilled water. Transfer the solutions to beakers or jars. Each solution contains 100 p.p.b. (parts per billion) phenol.
2. Stir the five portions on the mechanical stirrer for several minutes.
3. Make up a carbon suspension by weighing 0.250 gram of carbon and transferring it to a dry 250-ml. beaker. Add 100 ml. of phenol-free water (107). Place on the mechanical stirrer and stir continuously at 250 r.p.m. until all of the transfers to be made in Step No. 4 are complete. One milliliter of this suspension contains 0.0025 gram of carbon.
4. Add from a pipette 1, 2, 3, 4 and 8 ml., respectively, of the carbon suspension to each of the five phenol solutions prepared in Step No. 1. In order to avoid errors, allow the remaining contents of the pipette to discharge back into the suspension between each transfer of carbon. The mixtures will contain 2.5, 5.0, 7.5, 10.0 and 20.0 p.p.m. carbon, respectively.
5. Stir the mixtures for 1 hr. on the mechanical stirrer.
6. Filter the five mixtures through double No. 40 Whatman filter papers, with a Büchner funnel and the aid of suction. Discard the first 100 ml. Use new papers and a clean flask for each. Wash the papers with small amounts of phenol-free water (107).
7. Prepare standards from the standard phenol solution (109) by adding with a burette 0, 0.5, 1.0, 2.0, 3.0, 4.0, 5.0, 6.0, 8.0 and 10.0 ml., respectively, to 100-ml. Nessler tubes and fill

up to the mark with phenol-free water (107). These standards contain 0, 5, 10, 20, 30, 40, 50, 60, 80 and 100 p.p.b. phenol.

8. After the stirring in Step No. 5 has proceeded for about ½ hr., prepare diazotized sulfanilic acid by adding 20 ml. of sulfuric acid (63) to 100 ml. of sulfanilic acid (103). Mix and add 100 ml. of freshly prepared sodium nitrite solution (104) a few ml. at a time, mixing with a swirling motion. Stopper the mixture and pack in ice for ½ hr. before using.
9. Place 100 ml. of the previously filtered samples (Step No. 6) in Nessler tubes.
10. To each standard and sample add 10 ml. of the diazotized sulfanilic acid.
11. Stopper the tubes with clean dry rubber stoppers and mix by tilting the rack (holding the tubes) twice to a horizontal position.

Note.—The rack should be capable of holding two rows of 12 tubes each, with holes in the bottom of the rack large enough not to obstruct vision, but not large enough to allow the tubes to pass through.

12. Remove the stoppers and add 5 ml. of 8 per cent sodium hydroxide (106) to each tube.
13. Stopper the tubes again with dry rubber stoppers and tilt the rack to a horizontal position ten times.
14. Allow to stand for 5 min. and match the color of the samples with the standards. Record the p.p.b. phenol remaining in each. These values, each subtracted from 100, give the p.p.b. phenol removed by the various amounts of carbon.

Note.—The results may be plotted on 2-cycle, semilogarithmic graph paper, plotting the p.p.b. phenol on the logarithmic scale and the p.p.m. carbon on the uniform scale.

Jar Test for Coagulation.

Reagents: Sec. II (114).

1. Measure 1-liter quantities of the water to be tested into a series of glass jars.
2. Attach to a stirring device.

3. Add progressive volumes of the chemical solution (114) to each to cover the range of chemical dosage expected.
4. Mix the chemical rapidly and follow by a period of gentle stirring (about 40 r.p.m. in 1.5- to 2.0-liter jar).
5. Observe the character of the floc and its settling rate.
6. Select the minimum dosage giving the best floc and settling characteristics.

Note.—The coagulation period selected in Step No. 4 should correspond to the period employed in the treatment plant.

DIVISION II

SEWAGE

Note.—Numbers inclosed in parentheses, thus (3), refer to numbered reagents and solutions in Section II.

Total Solids.

1. Clean an evaporating dish and place it in a 103°C. oven for 1 hr., or if the loss on ignition determination is also to be made, ignite to low red heat in the muffle furnace or over a burner. (A platinum dish is preferred, but others may be used.)
2. Place the dish in a desiccator until cool, then weigh.
3. Place the weighed dish on a steam or water bath.
4. Thoroughly mix the sample and measure out 100 ml. into a 100-ml. graduate. (Care must be taken to keep the solids in suspension while measuring.)
5. Transfer the sample to the dish. Rinse the graduate several times with small portions of distilled water and add the rinsings to the dish. Be sure that all suspended matter is transferred.
6. After the sample is evaporated, dry the dish and residue in the 103°C. oven for 1 hr., cool in the desiccator and weigh.

Calculations.

$$\frac{\text{Increase in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. total solids}$$

Note.—The results may be checked by testing duplicate portions.

Example.

Sample portion	No. 1
Weight of dish and residue (100 ml.)	48.3424 gm.
Weight of dish	48.2340 gm.
Weight of solids	0.0884 gm.

Total solids:

$$\frac{0.0884 \times 1{,}000{,}000}{100} = 884 \text{ p.p.m.}$$

Loss on Ignition (Volatile Matter) and Fixed Residue.

1. Place the evaporating dish containing the residue from the total-solids determination in the muffle furnace or ignite over a burner at low red heat until the ash is white or nearly so. (Not over 1 hr. should be required.)
2. Allow the dish to cool and moisten the ash with a few drops of distilled water.
3. Dry in the 103°C. oven for 1 hr., cool in a desiccator and weigh.

Calculations.

$$\frac{\text{Loss in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. loss on ignition}$$

P.p.m. total solids — p.p.m. loss on ignition = p.p.m. fixed residue

Example.

Weight of dish and residue (100 ml.)	48.3424 gm.
Weight of dish and ash	48.3130 gm.
Loss in weight	0.0294 gm.

$$\text{Loss on ignition} = \frac{0.0294 \times 1{,}000{,}000}{100} = 294 \text{ p.p.m.}$$

Fixed residue = 884 — 294 = 590 p.p.m.

Note.—Part of the loss on ignition may be due to partial decomposition of mineral matter. Therefore this test is not a true determination of organic matter. See discussion on Sludge Ignition, page 181.

Suspended and Dissolved Solids, Gooch Crucible Method.

Reagents: Sec. II (1).

1. Place a Gooch crucible in a suction flask and pour into it about 25 ml. of asbestos emulsion (1).
2. Apply the suction gently until the mat is formed.
3. Wash with distilled water until no fibers of asbestos pass through the filter.
4. Dry the crucible in the 103°C. oven and ignite, in a muffle furnace or over a burner, at low red heat.
5. Allow the dish to cool and moisten the mat with distilled water.

6. Dry in the 103°C. oven for 1 hr., cool in the desiccator and weigh. (Step Nos. 4 and 5 may be omitted if the loss on ignition of suspended solids is not to be determined.)
7. Measure 100 ml. of the well-mixed sewage by means of a graduate. (It may be necessary to use a smaller portion than 100 ml. for raw sewage or other wastes high in suspended matter.) Keep the solids in suspension while measuring.
8. Filter the sample portion through the prepared Gooch crucible and follow with several distilled-water rinsings of the graduate. Be sure that all suspended matter is transferred to the crucible.
9. Dry in the 103°C. oven for 1 hr., cool in the desiccator and weigh.

Calculations.

$$\frac{\text{Increase in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. suspended solids}$$

P.p.m. total solids — p.p.m. suspended solids = p.p.m. dissolved solids

Example.

Weight of crucible and solids (100 ml.).....	15.5999 gm.
Weight of crucible.....	15.5817 gm.
Weight of solids.....	0.0182 gm.

Suspended solids:

$$\frac{0.0182 \times 1{,}000{,}000}{100} = 182 \text{ p.p.m.}$$

Dissolved solids = 884 — 182 = 702 p.p.m.

Loss on Ignition, of Suspended and Dissolved Solids, Gooch Crucible Method.

1. Ignite the crucible containing the residue from the suspended-solids determination at low red heat.
2. Allow the crucible to cool and moisten the ash with a few drops of distilled water.
3. Dry at 103°C. for 1 hr., cool in a desiccator and weigh.

Calculations.

$$\frac{\text{Loss in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. loss on ignition}$$

P.p.m. loss on ignition of total solids — p.p. m. loss on ignition of suspended solids = p.p.m. loss on ignition of dissolved solids

Example.

Weight of crucible and solids (100 ml.)	15.5999 gm.
Weight of crucible and ash	15.5889 gm.
Loss in weight	0.0110 gm.

$$\text{Loss on ignition, suspended solids} = \frac{0.0110 \times 1{,}000{,}000}{100} = 110 \text{ p.p.m.}$$

Loss on ignition, dissolved solids = 294 — 110 = 184 p.p.m.

Suspended and Dissolved Solids, Filtration Method.

1. Filter a portion of the sewage through a filter paper.
2. Follow the method given for Total Solids using 100 ml. of the above filtrate.

Calculations.

$$\frac{\text{Increase in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. dissolved solids}$$

P.p.m. total solids — p.p.m. dissolved solids = p.p.m. suspended solids

Example.

Weight of dish and residue (100 ml.)	48.3242 gm.
Weight of dish	48.2540 gm.
Increase in weight	0.0702 gm.

Dissolved solids:

$$\frac{0.0702 \times 1{,}000{,}000}{100} = 702 \text{ p.p.m.}$$

Suspended solids = 884 — 702 = 182 p.p.m.

Loss on Ignition, Suspended and Dissolved Solids, Filtration Method.

1. Ignite the dish and residue from the dissolved-solids determination to low red heat.
2. Cool and moisten the residue with a few drops of distilled water.
3. Dry at 103°C. for 1 hr., cool in a desiccator and weigh.

Calculations.

$$\frac{\text{Loss in weight (gm.)} \times 1{,}000{,}000}{\text{Ml. of sample}} = \text{p.p.m. loss on ignition of dissolved solids}$$

P.p.m. loss on ignition of total solids − p.p.m. loss on ignition of dissolved solids = p.p.m. loss on ignition of suspended solids

Example.

Weight of dish and residue (100 ml.)	48.3242 gm.
Weight of dish and ash	48.3058 gm.
Loss in weight	0.0184 gm.

$$\text{Loss on ignition of dissolved solids} = \frac{0.0184 \times 1{,}000{,}000}{100} = 184 \text{ p.p.m.}$$

Loss on ignition of suspended solids = 294 − 184 = 110 p.p.m.

Settleable Solids in Sewage and Activated Sludge.

1. Fill an Imhoff cone to the liter mark with the thoroughly mixed sewage. (For activated sludge a liter graduate should be used in place of the cone.)
2. Allow the solids to settle quietly for 2 hr. (A 30-min. period is used for activated sludge.)
3. Read the volume of solids settled in the tip of the cone or the bottom of the graduate.

Calculations.

Report the results as ml. of settleable solids per liter.

Solids in Activated Sludge.

Reagents: Sec. II (102).

1. Fill a 500-ml. graduated cylinder to the 500-ml. mark with the activated sludge (or the aeration-tank contents).
2. Add 1 ml. of ferric chloride solution (102) and mix rapidly.
3. Stir slowly by means of a rod for a few minutes.
4. Weigh a 12.5-cm. filter paper to the nearest 0.1 gram on the trip scale.
5. Place the filter paper in a Büchner funnel (126-mm. inside diameter) and filter the sludge, applying the vacuum as required.
6. Before the sludge is dry, scrape the adhering sludge from the sides of the funnel onto the paper by means of a spatula.

7. Carefully remove the paper and sludge from the funnel, place in a 103°C. oven for 1 hr. and weigh to the nearest 0.1 gram.

Calculations.

[Weight of paper and sludge (gm.) — weight of paper — 0.1] × 0.2 = per cent solids

[Weight of paper and sludge (gm.) — weight of paper — 0.1] × 2,000 = p.p.m. suspended solids

$$\frac{\text{Settleable solids in ml. per liter} \times 1{,}000}{\text{P.p.m. suspended solids}} = \text{sludge index}$$

Note.—This value of sludge index is the volume in ml. as sludge occupied by 1 gram of suspended solids after settling 30 min. This value was proposed by F. W. Mohlman in *Sewage Works Journal*, page 119, January, 1934.

Centrifuge Test.

1. Fill two 15-ml. A.P.I. standard centrifuge tubes to the mark with the well-mixed mixed-liquor or return activated sludge.
2. Spin in a clinical centrifuge at about 2,500 r.p.m. for exactly 15 min.
3. Read the volume of sludge in the tubes in per cent.

Note.—The percentage volume may be roughly converted to p.p.m. by multiplying by 1,000. This may be made more accurate by producing a curve from suspended-solids and centrifuge tests.

Alkalinity.

Reagents: Sec. II (2), (3), (4).

Discussion: Sec. III, page 140.

1. Pipette 100 ml. of the sample into one Erlenmeyer flask and the same quantity of distilled water into another.
2. Add 3 drops of phenolphthalein (2) to each.
3. If the sample becomes pink, add 0.02N sulfuric acid (3) from a burette until the pink color just disappears and record the number of ml. of acid used.
4. Add 3 drops of methyl orange (4) to each flask.
5. If the sample becomes yellow, add 0.02N sulfuric acid until the first difference in color is noted when compared with the distilled water. The end point is a slight orange tinge. Record the ml. of acid used.

Calculations.

Total alkalinity as $CaCO_3$ = total ml. acid used × 10

Differentiation of alkalinities due to hydroxide (OH), normal carbonate (CO_3) and bicarbonate (HCO_3) is made as explained below.

Let P = the ml. of 0.02N acid used for the titration with phenolphthalein and T = the ml. of acid used for the total titration (phenolphthalein plus methyl orange).

There are five possible conditions as follows:

1. $P = T$
   Hydroxide (p.p.m.) = $P \times 10$
2. $P > \frac{1}{2}T$
   Hydroxide (p.p.m.) = $(2P - T) \times 10$
   Normal carbonate (p.p.m.) = $2(T - P) \times 10$
3. $P = \frac{1}{2}T$
   Normal carbonate (p.p.m.) = $T \times 10$
4. $P < \frac{1}{2}T$
   Normal carbonate (p.p.m.) = $2P \times 10$
   Bicarbonate (p.p.m.) = $(T - 2P) \times 10$
5. $P = 0$
   Bicarbonate (p.p.m.) = $T \times 10$

All above results are in terms of $CaCO_3$.

For illustrative example see pages 9 and 143.

Acidity.

Reagents: Sec. II (2), (5).

Discussion: Sec. III, page 143.

1. Pipette 100 ml. of the sample into one Erlenmeyer flask and the same quantity of distilled water into another.
2. Add 3 drops of phenolphthalein (2) to each.
3. Add 0.02N sodium hydroxide (5) from a burette to the sample until the first permanent pink color appears as compared with the distilled water. Record the ml. of sodium hydroxide used.

Calculations.

Ml. of 0.02N NaOH × 10 = p.p.m. total acidity expressed in terms of $CaCO_3$

Hydrogen-ion Concentration (pH).

Discussion: Sec. III, page 144.

Note.—There are a number of different types of colorimetric pH outfits on the market each requiring a slightly different procedure in making the test. The general method below is adapted to most of these outfits.

1. Place 10 ml. of the sample into each of the two or three tubes provided.
2. To one tube add the designated quantity of indicator solution. (The other tubes do not receive the indicator.)
3. Place the tubes in the comparator in such a manner that the color standards are opposite the tubes not containing the indicator. The color comparison must be made by looking through the same thickness of liquid having the same color and turbidity as the sample.
4. Compare the colors and read the pH of the tube having a color nearest that of the sample.

Note.—Electrometric pH meters cover the entire range of pH values and are better adapted for the determination of pH of sewage than colorimetric outfits. Special directions for their use are furnished by the manufacturers.

Dissolved Oxygen, Winkler Method.

See page 38.

Dissolved Oxygen, Rideal-Stewart Modification.

See page 39.

Dissolved Oxygen of Activated-sludge Mixtures.

Reagents: Sec. II (74).

1. Fill to overflowing a 2-liter glass-stoppered bottle with the activated-sludge or aeration-tank mixture.
2. Immediately add 10 ml. of a 10 per cent solution of copper sulfate (74), insert the stopper without entrainment of air bubbles and mix by inverting the bottles several times.
3. Allow to settle until a clear supernatant liquor is obtained.
4. Siphon a portion of the clear liquor into an 8-oz. glass-stoppered bottle. Allow the liquor to overflow from the bottle to flush out the air.
5. Make the dissolved-oxygen determination as given on page 39.

Biochemical Oxygen Demand.

Reagents: Sec. II (70), (71), (72).

Discussion: Sec. III, page 176.

1. Clean the desired number of 8-oz. glass-stoppered bottles by filling them with cleaning solution (70). Allow them to

stand for some time and rinse with tap water until the solution is entirely removed.

Note.—If an air incubator is to be used, it will be necessary to fit each bottle with a water seal made of heavy rubber tubing, or special B.O.D. bottles may be used which have a seal provided. Seals will not be necessary if the bottles are submerged in a water incubator.

2. Fill one bottle completely with diluting water (71), insert the stopper tightly and place in incubator.
3. Make up dilutions for incubation by either (*a*) or (*b*).

Note.—B.O.D. dilutions of sewage samples which have been partly or completely sterilized by chlorination or other means should be seeded. This may be accomplished by adding 1 ml. of settled sewage to each of the 8-oz. dilutions and to the 8-oz. bottle of diluting water.

a. For dilutions to contain 1 per cent or more of sample. Measure the capacity of the bottles (72). Fill each about halfway with diluting water (71) by means of a siphon. Add the quantity of well-mixed sewage or waste necessary to make the desired dilution and then fill completely with the diluting water. Insert the stopper tightly without entraining air bubbles and place in incubator.

b. For dilutions to contain less than 1 per cent of the sample. Siphon about 500 ml. of diluting water into a liter graduate. (The graduate must be clean.) Add the quantity of well-mixed sewage or waste necessary to make the desired dilution and fill to 1,000 ml. with diluting water. Mix well with a plunger type stirring rod, avoiding the entrainment of air. Siphon the well-mixed dilution into one of the 8-oz. incubation bottles, tightly stopper and place in incubator.

4. Proceed by either (*a*) or (*b*).

a. For dilutions containing less than 5 per cent of the sample. Incubate the diluting water and the dilutions at 20°C. (±1°) for 5 days. At the end of the incubation period make a dissolved-oxygen determination on the diluting water and on each dilution, using the Winkler method (page 38).

Calculations.

$$\frac{(\text{P.p.m. D.O. in diluting water} - \text{p.p.m. D.O. in dilution}) \times 100}{\text{Per cent of sample in the dilution}} = \text{p.p.m. 5-day B.O.D.}$$

b. For dilutions to contain more than 5 per cent of the sample. Fill two bottles with diluting water. Make up two dilutions for each percentage dilution using the method given in Step No. 3*a*. Place one diluting-water sample and one sample of each percentage dilution in the 20°C. incubator. Make the dissolved-oxygen determinations at once on the other samples. After 5 days' incubation make the dissolved-oxygen determinations on the incubated samples. The difference in the D.O. of the diluting water before and after incubation is the correction for the B.O.D. of that water.

Calculations.

Let A equal the D.O. of the dilution before incubation, B equal the D.O. after incubation and C equal the correction as determined above.

$$\frac{(A - B - C) \times 100}{\text{Per cent of sample in dilution}} = \text{p.p.m. 5-day B.O.D.}$$

Oxygen Consumed.

Reagents: Sec. II (63), (64), (65).
Discussion: Sec. III, page 173.

1. Measure 50 ml. of the well-mixed sewage into a 250-ml. Erlenmeyer flask using a 50-ml. graduate. (For very strong sewages or trade wastes use a smaller portion.)
2. Rinse the graduate well with distilled water. Add the rinsings to the flask and finally add distilled water to give a total volume of about 100 ml.
3. In a second flask place 100 ml. of distilled water. This water is to be used as a check.
4. To each add 10 ml. of dilute sulfuric acid (63) and 10 ml. of standard potassium permanganate solution (65).
5. Place each flask in a boiling-water bath and digest for exactly 30 min. Be sure that the water in the bath is at all times above the surface of that in the flasks. Should the permanga-

nate color become faint, repeat the digestion using a larger quantity of permanganate.

6. Add 10 ml. of standard ammonium oxalate solution (64) to each flask.
7. Add drop by drop from a burette standard permanganate until the first permanent pink color is obtained and record the ml. used in each case.

Calculations.

Let K = total ml. of $KMnO_4$ used for the sample including that added before digestion
N = total ml. of oxalate used for the sample
k = ml. $KMnO_4$ used for the distilled water
n = ml. oxalate used for the distilled water

$$\frac{[(K - N) - (k - n)] \times 100}{\text{Ml. of sample}} = \text{p.p.m. oxygen consumed}$$

Example.

Total volume sample used	50 ml.
Total $KMnO_4$ added to sample (K)	24.4 ml.
Total oxalate added to sample (N)	10.0 ml.
Total $KMnO_4$ added to distilled water (k)	11.2 ml.
Total oxalate added to distilled water (n)	10.0 ml.

$$\frac{[(24.4 - 10.0) - (11.2 - 10.0)] \times 100}{50} = 26.4 \text{ p.p.m.}$$

Relative Stability.

Reagents: Sec. II (73).
Discussion: Sec. III, page 178.

1. Fill completely to overflowing a 6- or 8-oz. glass-stoppered bottle with the sample to be tested.
2. Add 0.5 ml. of a solution of methylene blue (73) by means of a pipette, extending the tip just below the surface of the liquid in the bottle.
3. Insert the stopper so that no air bubbles are entrained beneath it.
4. Keep the bottle where the temperature is nearly constant and as near 20°C. as possible.

5. Observe the bottle at least once each day and record the time necessary for the disappearance of the color.

Calculations.

Read the per cent stability from the following table:

Days Required for Disappearance of Color	Relative Stability Per Cent
0.5	11
1.0	21
2.0	37
3.0	50
4.0	60
5.0	67
6.0	75
7.0	80
8.0	84
9.0	87
10.0	90
12.0	95

Ammonia Nitrogen, Direct Nesslerization.

Reagents: Sec. II (44), (45), (46), (47), (55), (74).
Discussion: Sec. III, page 171.

1. Place 100 ml. of the sample in a Nessler tube and add 1 ml. of a 10 per cent solution of copper sulfate (74).
2. Mix by rotating and add 1 ml. of 50 per cent sodium hydroxide (55).
3. Mix again and allow to settle.
4. Pipette a measured portion of the clear supernatant liquor (5 to 25 ml. depending upon the ammonia content) into a second Nessler tube and dilute to 100 ml. with ammonia-free water (44).
5. If permanent ammonia standards are available (45) proceed to Step No. 6. If not, make up temporary standards by adding 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.7, 2.0, 2.5 and 3.0 ml. of standard ammonium chloride (46) to 100-ml. Nessler tubes and dilute to the mark with ammonia-free water.
6. Add 2 ml. of Nessler reagent (47) to the sample (and to each temporary standard, if used).
7. After 10 min., compare the colors and record the standard having a color nearest to that of the sample.

Calculations.

a. Using permanent standards

$$\frac{\text{Mg. N in permanent standard} \times 1{,}000}{\text{Ml. portion used in Step No. 4}} = \text{p.p.m. ammonia nitrogen as N}$$

b. Using temporary standards

$$\frac{\text{Ml. } NH_4Cl \text{ in standard} \times 10}{\text{Ml. portion used in Step No. 4}} = \text{p.p.m. ammonia nitrogen as N}$$

Albuminoid Nitrogen.

Reagents: Sec. II (44), (45), (46), (47), (48).
Discussion: Sec. III, page 171.

1. Measure 50 ml. of the well-mixed sewage sample and 50 ml. of distilled water into a 500-ml. Kjeldahl flask. (For a water sample use 500 ml. without dilution.)
2. Connect to the distillation apparatus and boil until the distillate is free from ammonia as shown by adding a few drops of Nessler reagent (47) to a small portion of the distillate. The volume will be reduced about one-half.
3. Cool the residue. Bring the volume up to about 300 ml. with ammonia-free water (44) and add 50 ml. of alkaline potassium permanganate (48). (This oxidizes the nitrogenous organic matter, liberating the nitrogen as ammonia.)
4. Reconnect to the distillation apparatus and distill until almost 200 ml. of distillate is collected in a 200-ml. volumetric flask. Distillation should be at a rate of 6 to 10 ml. per minute.
5. Make the distillate up to the mark with ammonia-free water and mix well.
6. Pipette a measured portion into a 100-ml. Nessler tube and make up to the mark with ammonia-free water. The portion used can only be determined by trial.
7. If permanent ammonia standards (45) are available, proceed to Step No. 8. If not, make up temporary standards by adding 0.2, 0.4, 0.6, 0.8, 1.0, 1.7, 2.0, 2.5 and 3.0 ml. of standard ammonium chloride (46) to 100-ml. Nessler tubes and making up to the mark with ammonia-free water.
8. Add 2 ml. of Nessler reagent to the sample (and to each temporary standard, if used).

9. After standing 10 min., compare the colors and record the standard having a color nearest that of the sample.

Calculations.

a. Using permanent standards

$$\frac{\text{Mg. N in permanent standard} \times 200{,}000}{\text{Ml. of sample Step No. 1} \times \text{ml. of portion Step No. 6}} = \text{p.p.m. albuminoid nitrogen as N}$$

b. Using temporary standards

$$\frac{\text{Ml. standard } NH_4Cl \times 2{,}000}{\text{Ml. of sample Step No. 1} \times \text{ml. of portion Step No. 6}} = \text{p.p.m. albuminoid nitrogen as N}$$

Organic Nitrogen, Kjeldahl Method.

Reagents: Sec. II (2), (44), (45), (46), (47), (55), (74).
Discussion: Sec. III, page 170.

1. Measure 50 ml. of the well-mixed sewage sample and 50 ml. of distilled water into a 500-ml. Kjeldahl flask. (For a water sample use 500 ml. of sample without dilution.)
2. Connect to the distillation apparatus and boil until the distillate is free from ammonia as shown by adding a few drops of Nessler reagent (47) to a small portion of the distillate. The volume will be reduced about one-half.
3. Cool the residue and add 1 ml. of 10 per cent copper sulfate (74) and 10 ml. of concentrated sulfuric acid. (For a water sample use 5 ml. of acid and no copper sulfate.)
4. Digest in the fume hood until white fumes of sulfuric acid are obtained and until the solution is colorless or light straw color and free from black carbon particles.
5. Cool and bring volume up to about 250 ml. with ammonia-free water (44).
6. Add a few drops of phenolphthalein (2) and make alkaline with 50 per cent sodium hydroxide (55).
7. Add a few boiling chips, reconnect to the distillation apparatus and distill until almost 200 ml. of distillate is collected in a 200-ml. volumetric flask. (The apparatus should be free from ammonia before this distillation is started.)
8. Make up the distillate to the mark with ammonia-free water and mix thoroughly.

9. Pipette a measured portion (5 ml. or more) into a 100-ml. Nessler tube and make up to the mark with ammonia-free water. The portion used will vary and must be determined by trial.
10. If permanent ammonia standards (45) are available, proceed to Step No. 11. If not, make up temporary standards by adding 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.7, 2.0, 2.5 and 3.0 ml. of standard ammonium chloride (46) to 100-ml. Nessler tubes and making up to the mark with ammonia-free water.
11. Add 2 ml. of Nessler reagent (47) to the sample (and to each temporary standard, if used).
12. After standing 10 min., compare the colors and record the standard having a color nearest that of the sample.

Calculations.

a. Using permanent standards

$$\frac{\text{Mg. N in permanent standard} \times 200{,}000}{\text{Ml. of sample Step No. 1} \times \text{ml. of portion Step No. 9}} = \text{p.p.m. organic nitrogen as N}$$

b. Using temporary standards

$$\frac{\text{Ml. of standard } NH_4Cl \times 2{,}000}{\text{Ml. of sample Step No. 1} \times \text{ml. of portion Step No. 9}} = \text{p.p.m. organic nitrogen as N}$$

Nitrite Nitrogen.

Reagents: Sec. II (15), (50), (51), (52).
Discussion: Sec. III, page 172.

1. If the sample is colored or turbid, clarify 150 ml. by adding 2 ml. of aluminum hydroxide (15). Heat to boiling and filter.
2. Place a measured portion of the filtrate (10 to 50 ml., depending upon the nitrite content) into a 100-ml. Nessler tube and make up to the mark with distilled water.
3. If permanent standards are available, proceed to Step No. 4. If not, make up temporary standards by adding 0.2, 0.4, 0.6, 0.8, 1.0, 1.4, 1.7, 2.0 and 2.5 ml. of standard sodium nitrite (50) in 100-ml. Nessler tubes and make up to the mark with nitrite-free water.
4. Add 2 ml. of sulfanilic acid (51) and 2 ml. of a-naphthylamine (52) to the sample (and to each temporary standard, if used).

5. Mix and allow to stand 10 min.
6. Compare the colors and record the standard having a color nearest to that of the sample.

Calculations.

a. Using permanent standards

$$\frac{\text{Mg. N in permanent standard} \times 1{,}000}{\text{Ml. portion used in Step No. 2}} = \text{p.p.m. nitrite nitrogen as N}$$

b. Using temporary standards

$$\frac{\text{Ml. standard } NaNO_2 \times 0.5}{\text{Ml. portion used in Step No. 2}} = \text{p.p.m. nitrite nitrogen as N}$$

Nitrate Nitrogen, Phenoldisulfonic Acid Method.

Reagents: Sec. II (54), (55), (56).
Discussion: Sec. III, page 172.

1. Filter 30 to 35 ml. of the sample through a filter paper.
2. Evaporate 25 ml. of the filtrate to dryness on a water bath. (Use a smaller amount if the nitrate content is high.)
3. Moisten the residue with 1 ml. of phenoldisulfonic acid (54).
4. Dilute to about 20 ml. with distilled water.
5. Add a 50 per cent solution of sodium hydroxide (55) until the maximum yellow color is developed. (Not more than 5 to 6 ml. of sodium hydroxide will be required.)
6. Filter into a 100-ml. Nessler tube, rinse the dish and paper with distilled water. Add the filtered rinsings to the filtrate and make up to the mark with distilled water.
7. If permanent standards are available, proceed to Step No. 8. If not, make up temporary standards by placing 0.2, 0.4, 0.6, 0.8, 1.0, 1.5, 2.0, 3.0, 4.0 and 5.0 ml. of standard sodium nitrate solution (56) in 100-ml. Nessler tubes and adding 2 ml. of 50 per cent sodium hydroxide.
8. Dilute to the mark with distilled water.
9. Compare the colors and record the standard having a color nearest to that of the sample.

Calculations.

a. Using permanent standards

$$\frac{\text{Mg. N in permanent standard} \times 1{,}000}{\text{Ml. of sample used in Step No. 2}} = \text{p.p.m. nitrate nitrogen as N}$$

b. Using temporary standards

$$\frac{\text{Ml. of standard } NaNO_3 \times 10}{\text{Ml. of sample used in Step No. 2}} = \text{p.p.m. nitrate nitrogen as N}$$

Chlorine Demand.

Reagents: Sec. II (60), (75).
Discussion: Sec. III, page 168.

1. Place 200 ml. of the well-mixed sewage in each of ten 8-oz. bottles.
2. Add 0.5 ml. of chlorine water (75) to the first bottle, 1.0 ml. to the second, 1.5 to the third and so on, increasing by 0.5 ml. until all ten bottles have been treated.
3. Shake each bottle gently and allow to stand for 30 min.
4. Add a crystal of potassium iodide and 1 ml. of starch solution (60) to each bottle and mix. Record the ml. of chlorine water in the bottle containing the least amount of chlorine water which shows a blue color.

Calculations.

Ml. of chlorine water in first bottle to show a blue color $\times$ 5 = p.p.m. chlorine demand

Residual Chlorine, Orthotolidine Method.

Reagents: Sec. II (66), (67).
Discussion: Sec. III, page 169.

1. Place 100 ml. of sample in a Nessler tube. If the temperature of the sample is below 20°C., bring it up to this temperature by immersing the tube in warm water.
2. Add 1 ml. of orthotolidine solution (66) and mix.
3. A yellow color indicates the presence of residual chlorine.
4. For a quantitative estimation, allow the sample to stand for 5 min. in a dark place and compare the color with permanent standards (67) by looking down through the tubes against a white background illuminated with good light. Record the standard having a color nearest to that of the sample.

Residual Chlorine, Starch-iodide Method.

Reagents: Sec. II (60), (68).
Discussion: Sec. III, page 169.

1. Place 200 ml. of the sample in an Erlenmeyer flask. Chill to 20°C. or lower by immersing the flask in a bath of cold water.
2. Add about 1 gram of potassium iodide crystals and 1 ml. of concentrated hydrochloric acid.
3. Add 1 ml. of starch solution (60). A blue color indicates the presence of chlorine.
4. For a quantitative estimation, titrate the solution with 0.001N sodium thiosulfate solution (68) until the blue color just disappears. Record the ml. of thiosulfate used.

Calculations.

Ml. of 0.001N thiosulfate × 0.1773 = p.p.m. residual chlorine

Chlorides.

Reagents: Sec. II (2), (4), (13), (14), (15).
Discussion: Sec. III, page 163.

1. Pipette 50 ml. of the sample into an evaporating dish.
2. Place 50 ml. of distilled water in a second dish for a comparison of color.
3. Add 1 ml. of potassium chromate indicator (13) to the sample and to the distilled water.
4. Add standard silver nitrate (14), a few drops at a time, from a burette to the sample until the first permanent reddish color is obtained. Record the ml. of silver nitrate used.
5. If more than 7 or 8 ml. of silver nitrate are required, the entire procedure must be repeated using a smaller sample diluted to 50 ml. with distilled water.

Calculations.

$$\frac{(\text{Ml. of } AgNO_3 - 0.2) \times 500}{\text{Ml. of sample}} = \text{p.p.m. chloride radical (Cl)}$$

Precautions.—If the sample contains hydrogen sulfide, slightly acidify a 50-ml. portion in an evaporating dish with sulfuric acid. Boil, cool and neutralize by adding a saturated solution of sodium carbonate, drop by drop, until no further effervescence occurs.

If the sample is highly colored, it should be decolorized by shaking with washed aluminum hydroxide (15) and filtering.

If it is highly acid, add 10 per cent sodium carbonate solution until it is slightly alkaline to methyl orange (4).

If it is highly alkaline, add dilute sulfuric acid until it is just acid to phenolphthalein (2).

Fats (Grease).

Reagents: Sec. II (4), (76).

1. Place 500 ml. of the sample in an evaporating dish and evaporate over a water bath until the volume is reduced to about 50 ml.
2. Scrape the solids from the sides into the liquid with a glass rod, add a few drops of methyl orange (4) and 1N hydrochloric acid (76) until the solution is red.
3. Evaporate to dryness on the water bath and dry for 30 min. in the 103°C. oven.
4. Dry, cool and weigh a 150-ml. Soxhlett flask.
5. Bring about 50 ml. of ether to boiling on a water bath from which the flame has been removed.
6. Pour the ether over the solids in the evaporating dish and rub in well with a stirring rod.
7. Decant the ether through a dry filter into the Soxhlett flask.
8. Repeat the extractions twice using 20-ml. portions of ether.
9. Evaporate the ether on the bath, dry the flask at 103°C. and cool in a desiccator.
10. Weigh the flask and extract.

Calculations.

Difference in weight (gm.) × 2,000 = p.p.m. fat

Hydrogen Sulfide (H_2S).

Reagents: Sec. II (59), (60), (69).

Discussion: Sec. III, page 179.

1. Siphon 500 ml. of the sample into a graduated cylinder.
2. Pipette 10 ml. of 0.025N iodine solution (69) into each of two Erlenmeyer flasks.
3. Add about 1 gram of potassium iodide crystals to each.
4. Add 200 ml. of distilled water to one flask.
5. Siphon 200 ml. of the sample from the graduate into the other flask.
6. Titrate both the distilled-water blank and the sample with 0.025N sodium thiosulfate (59) using starch as an indicator (60) near the end of the titration. Record the ml. of thiosulfate used in each case.

Calculations.

Let S = ml. of sodium thiosulfate used for sample
and D = ml. of sodium thiosulfate used for distilled water

$$\frac{(S - D) \times 426}{\text{Ml. of sample}} = \text{p.p.m. hydrogen sulfide}$$

Moisture of Sewage Sludge.

Note.—For moisture in dried sludge, see page 73.

1. Ignite, cool in a desiccator and weigh a clean evaporating dish of about 50-ml. capacity.
2. Mix the sludge thoroughly and pour approximately 25 ml. into the dish.
3. Reweigh immediately, avoiding delay as the sludge changes in weight rapidly.
4. Evaporate on the water bath until dry.
5. Place in the 103°C. oven for at least 1 hr., cool in the desiccator and weigh.

Calculations.

$$\frac{\text{Loss in weight (gm.)} \times 100}{\text{Weight of wet sludge (gm.)}} = \text{per cent moisture}$$

Note.—Weighings to 0.01 gm. are sufficiently accurate.

Example.

Weight of dish and wet sludge	58.12 gm.
Weight of dish	33.75 gm.
Weight of wet sludge	24.37 gm.
Weight of dish and wet sludge	58.12 gm.
Weight of dish and dry sludge	35.24 gm.
Loss in weight	22.88 gm.

$$\text{Moisture} = \frac{22.88 \times 100}{24.37} = 93.9 \text{ per cent}$$

Solids, dry = 100 − 93.9 = 6.1 per cent

Volatile Matter of Sewage Sludge.

1. Ignite the dish containing the dry solids from the moisture determination at a low red heat in the furnace or over a burner until no black residue remains.

2. Cool, moisten with distilled water and dry in the 103°C. oven for 1 hr.
3. Cool in the desiccator and weigh.

Calculations.

$$\frac{\text{Loss in weight (gm.)} \times 100}{\text{Weight of dry solids (gm.)}} = \text{per cent volatile matter}$$

Example.

Weight of dish and dry solids	35.24 gm.
Weight of dish	33.75 gm.
Weight of dry solids	1.49 gm.

Weight of dish and dry solids	35.24 gm.
Weight of dish and ash	34.22 gm.
Loss in weight	1.02 gm.

$$\text{Volatile matter} = \frac{1.02 \times 100}{1.49} = 68.5 \text{ per cent}$$

Specific Gravity of Sewage Sludge.

1. Weigh an empty wide-mouthed glass-stoppered bottle or flask of about 8-oz. or more capacity to the nearest 0.1 gram.
2. Fill to overflowing with distilled water, insert the stopper, dry with a cloth and weigh.
3. Completely empty the bottle, fill to overflowing with the well-mixed sludge and insert the stopper.
4. Wash the sludge from the outside of the bottle, dry with a cloth and weigh.

Calculations.

$$\frac{\text{Weight of the sludge (gm.)}}{\text{Weight of distilled water (gm.)}} = \text{specific gravity of sludge}$$

Hydrogen-ion Concentration (pH) of Sewage Sludge, Colorimetric Method.

Discussion: Sec. III, page 144.

1. Place about 20 ml. of the sludge in a 100-ml. graduate or a similar tall cylinder and dilute to about five times its volume with freshly boiled and cooled distilled water.
2. Mix well and allow to settle.

Note.—The sludge may be clarified by centrifuging instead of using the procedure given in steps Nos. 1 and 2 above.

3. Place 10 ml. of the supernatant liquor into each of the two or three tubes provided with the pH apparatus.
4. To one tube add the designated quantity of indicator.
5. Place the tubes in the comparator in such a manner that the color standards are opposite the tubes not containing the indicator. The color comparison must be made by looking through the same thickness of liquid having the same color and turbidity as the sample.
6. Compare the colors and select the standard having a color nearest to that of the sample.

Hydrogen-ion Concentration (pH), Electrometric Method.

Discussion: Sec. III, page 144.

Specific directions for this method accompany the apparatus when shipped from the manufacturer. When used with sewage sludge, the sludge need not be diluted or clarified as is necessary with colorimetric equipment.

Fats (Grease), Sewage Sludge.

Reagents: Sec. II (4), (76).

1. Weigh a clean, dry evaporating dish to the nearest 0.1 gram.
2. Mix the sludge sample thoroughly and pour about 25 ml. into the weighed dish.
3. Reweigh rapidly and record the weight of sludge used. (This weighing may also be made to the nearest 0.1 gram.)
4. Add a few drops of methyl orange (4) and then add 1N hydrochloric acid (76) until the supernatant liquid is red.
5. Evaporate to dryness on a water bath and dry for 30 min. in the 103°C. oven.
6. Dry, cool and weigh a clean 150-ml. Soxhlett flask.
7. Bring about 50 ml. of ether to boiling on a water bath from which the flame has been removed.
8. Pour the ether over the solids in the dish and rub in well with stirring rod.
9. Decant the ether through a dry filter paper into the Soxhlett flask.

10. Repeat the extraction three times using about 25-ml. portions of ether each time.
11. Evaporate the ether on the bath with flame removed, dry the flask at 103°C. and cool in a desiccator.
12. Weigh the flask and extract.

Calculations.

$$\frac{\text{Gain in weight (gm.)} \times 100}{\text{Weight of sample (gm.)}} = \text{per cent fat (grease)}$$

Fertilizer Value of Sewage Sludge.

Discussion: Sec. III, page 180.

The fertilizer series includes the determination of humus (organic matter), phosphoric acid, nitrogen and potash. The sample should be carefully taken to represent the sludge thoroughly. It is customary to report fertilizer values on a dry basis and therefore it is necessary to precede them by a moisture determination if it is desired to know their significance in relation to the dry sludge. It is also necessary to grind the sludge (which can only be done on a dried sample) in order to ensure ready digestion in the phosphoric acid and nitrogen determinations and to make portions of the sample used representative of the entire sample.

Moisture of Sewage Sludge.

Note.—For moisture in wet sludge, see page 70.

1. Weigh an evaporating dish, add about 100 grams or more of the sludge and weigh again. The difference in weights will be the weight of the sample.
2. Evaporate over a water bath, dry in the oven at 103°C. for 2 hr., cool in a desiccator and weigh.

Note.—These weighings need be carried only to the nearest 0.1 gram.

Calculations.

$$\frac{\text{Loss in weight (gm.)} \times 100}{\text{Weight of sample (gm.)}} = \text{per cent moisture}$$

Sludge, Preparation for Analysis.

Grind the entire residue from the above moisture determination to a fine state and preserve in a tightly stoppered bottle. If the

quantity of sample is too large to be preserved, it may be reduced in size as follows: Place the entire ground sample in a pile, divide it into quarters and retain opposite quarters. Repeat the quartering until the remaining sample is of the desired size. The grinding may be omitted if humus is the only determination to be made or if the sample is a commercial sludge fertilizer which has already been dried and ground.

Humus (Organic Matter) of Sewage Sludge.

Reagents: Sec. II (17), (18).
Discussion: Sec. III, page 181.

1. Ignite, cool and weigh an evaporating dish.
2. Place about 2 grams of the prepared sample in the dish and reweigh.
3. Place in a 103°C. oven for 2 hr. or until the weight is constant, cool in a desiccator and weigh.
4. Ignite at a cherry red heat (about 900°C.) for 1 hr.
5. Cool in a desiccator and weigh.

Note.—In order to allow for an appreciable loss of CO_2 in the above ignition due to the decomposition of calcium carbonate, it is necessary to determine the amount of the latter compound as follows. This does not take into account the possible decomposition of other inorganic compounds which may be present in lesser amounts in the sludge.

6. Carefully add about 5 ml. of concentrated hydrochloric acid to the ash in the dish, warm gently, add about 50 ml. of distilled water and warm again.
7. Rinse into a beaker, add 10 ml. of 10 per cent ammonium chloride (17) and make alkaline to litmus with concentrated ammonium hydroxide.
8. Cover with a watch glass, warm gently for a few minutes and filter off the iron and aluminum hydroxides. Rinse the beaker and paper with small portions of distilled water and add the filtered rinsings to the filtrate.
9. Add slowly, with constant stirring, about 10 ml. of saturated ammonium oxalate (18), cover with a cover glass and set the beaker in a warm place for 30 min.
10. Filter the precipitate onto a quantitative filter paper and wash well with hot distilled water.
11. Ignite, cool and weigh a clean crucible.

12. Place the paper and precipitate in the crucible, dry and ignite at a cherry red heat in the muffle furnace or over a Meeker burner.
13. Cool in the desiccator and weigh. Repeat the ignition until the weight is constant.

Calculations.

$$\frac{\text{(Weight in Step No. 3 − weight in Step No. 5)} \times 100}{\text{Weight of dry sample}} = \text{per cent volatile matter}$$

Weight in Step No. 2 − weight of dish = weight of dry sample

Weight of crucible and residue in Step No. 13 − weight of crucible in Step No. 11 = weight of CaO

$$\frac{\text{Weight of CaO} \times 178.5}{\text{Weight of dry sample}} = \text{per cent } CaCO_3 \text{ in sample}$$

Per cent volatile matter − (0.44 × per cent $CaCO_3$) = per cent humus in sample

Phosphoric Acid (P_2O_5) in Sewage Sludge.

Reagents: Sec. II (2), (77), (78), (79).
Discussion: Sec. III, page 183.

1. Dry, cool and weigh a crucible and add about 2 grams of the sample prepared as given under Sludge Preparation, pages 73 and 74.
2. Dry in the 103°C. oven for 2 hr. or until constant weight, cool in a desiccator and weigh.
3. Transfer the dried sample to a Kjeldahl flask and add about 30 ml. of concentrated sulfuric acid and 10 grams of anhydrous sodium sulfate.
4. Boil in the fume hood until the organic matter is destroyed. Cool, rinse into a 200-ml. volumetric flask, make up to the mark with distilled water and mix thoroughly. The distilled water should be added carefully with constant mixing.
5. Filter a portion and pipette 50 ml. of the filtrate into a beaker.
6. Add 5 ml. of concentrated nitric acid and then add concentrated ammonium hydroxide until the precipitate which forms dissolves slowly on stirring.
7. Dilute to about 100 ml. and heat in a water bath to 55 to 60°C.

8. Add 20 to 25 ml. of freshly filtered molybdate solution (77) and allow the mixture to remain in the bath with occasional stirring for 30 min.
9. Decant the solution through a filter paper and wash the precipitate in the beaker twice with about 25-ml. portions of distilled water, filtering each washing through the same paper.
10. Transfer the entire precipitate to the paper and wash with distilled water until a portion of the filtrate gives a pink color upon the addition of phenolphthalein (2) and a drop of sodium hydroxide (78).
11. Transfer the precipitate and filter to a beaker and dissolve the precipitate in a small excess of 0.3238N sodium hydroxide (78). Record the ml. of hydroxide used.
12. Add a few drops of phenolphthalein and titrate the excess hydroxide with 0.3238N hydrochloric acid (79). Record the ml. of acid used.

Calculations.

$$\frac{(\text{Ml. } 0.3238\text{N NaOH} - \text{ml. } 0.3238\text{N HCl}) \times 0.4}{\text{Weight (gm.) of dry sample used in Step No. 2}} = \text{per cent } P_2O_5$$

Total Nitrogen in Sewage Sludge.

Reagents: Sec. II (44), (55), (80), (81), (82).
Discussion: Sec. III, page 183.

1. Dry, cool and weigh a crucible. Add about 2 grams of the sample prepared as directed under Sludge Preparation, pages 73 and 74.
2. Dry in the 103°C. oven for 2 hr. or until constant weight, cool in a desiccator and weigh.
3. Transfer the dried sample to a Kjeldahl flask, add 30 to 35 ml. of salicylic acid mixture (80) and shake until well mixed.
4. Allow to stand 30 min. with frequent shaking.
5. Add 5 grams of sodium thiosulfate crystals and heat gently for 5 min. in a hood.
6. Cool and add 10 grams of potassium sulfate crystals.
7. Heat gently in the hood until foaming ceases and then boil until a clear straw-colored liquor is obtained. (Usually not less than 2 hr.)

8. Cool, dilute with about 200 ml. of ammonia-free water (44) and add a few boiling chips.
9. Add sufficient sodium hydroxide (55) to make the solution strongly alkaline to phenolphthalein.
10. Steam out the distillation apparatus until free from ammonia and connect the Kjeldahl flask.
11. Place 50 ml. of 4 per cent boric acid (81) in an Erlenmeyer flask and arrange in such a manner that the tip of the condenser extends just below the solution in the flask.
12. Distill about 150 ml. of the mixture into the boric acid.
13. Add 3 drops of methyl orange (4) and titrate the boric acid mixture with 0.5N hydrochloric acid (82). Record the ml. of acid used.

Calculations.

$$\frac{\text{Ml. of 0.5N HCl} \times 0.7}{\text{Weight of dried sample in Step No. 2}} = \text{per cent nitrogen as N}$$

Per cent nitrogen as N $\times$ 1.217 = per cent nitrogen as NH_3

Potash in Sewage Sludge.

Reagents: Sec. II (24), (25).
Discussion: Sec. III, page 184.

1. Dry, cool and weigh an evaporating dish. Add about 10 grams of the sample prepared as directed under Sludge Preparation, pages 73 and 74. Dry in the 103°C. oven, cool in a desiccator and weigh.
2. Saturate the sample with concentrated sulfuric acid and ignite in a muffle furnace, or over a burner, at low red heat until the black carbon is completely burned.
3. Cool, add 5 ml. of concentrated hydrochloric acid and warm to dissolve the acid-soluble material.
4. Add about 50 ml. of distilled water and filter into an evaporating dish, rinsing the dish and paper with several portions of distilled water and adding the filtered rinsings to the filtrate.
5. Evaporate the filtrate on the water bath until it contains very little water.
6. Add 3 ml. of platinic chloride (24) and again evaporate almost to dryness. (The residue should not be completely

dry since, if dry, it will be difficult to extract in the next step. The residue will solidify on cooling.)

7. Add 25 ml. of 80 per cent alcohol (25) to the residue in the dish and heat on the water bath for several minutes.
8. Cool and decant the solution through a filter paper.
9. Repeat Steps No. 7 and 8 three times using about 15 ml. of alcohol each time. Discard the filtrates.
10. Dissolve the residue remaining in the dish and on the paper in hot water and wash into a beaker.
11. Add 5 ml. of concentrated hydrochloric acid and several strips of magnesium ribbon. (All of the magnesium should go into solution and a black precipitate of platinum should appear.)
12. Ignite, cool and weigh a clean crucible.
13. Filter the black precipitate onto a quantitative filter paper and wash the paper with distilled water. Be sure all of the precipitate is transferred to the paper.
14. Place the paper in the crucible and ignite.
15. Cool in a desiccator and weigh.

Calculations.

$$\frac{\text{Gain in weight (gm.)} \times 48.25}{\text{Weight of sample (gm.)}} = \text{per cent } K_2O \text{ in sample}$$

Carbon Dioxide in Sewage Gas.

Reagents: Sec. II (83).

Discussion: Sec. III, page 186.

1. Waste a portion of the gas to the air in order to clear the lines and to obtain a representative sample. If the gas is not piped to the laboratory, a sample may be collected at any convenient place on the gas domes or from the lines to the burners. It should be collected in a flat rubber gas bag capable of holding about 1 liter.
2. Raise the leveling tube and fill the measuring pipette completely with the liquid. (Mercury is preferred.)
3. Attach the bag or pipe line and draw about 100 ml. of the gas into the pipette by lowering the leveling tube.
4. Close the stopcock connecting the gas bag or gas line and carefully measure the volume of gas in the pipette. Let this

volume in ml. = A (The volume of gas in the pipette should always be measured by holding the level of the liquid in the leveling tube at the same elevation as that in the pipette.)

5. Open the connection to the potassium hydroxide (83) pipette and pass the gas into that pipette, allowing it to remain in contact with the solution for some time.
6. By lowering the leveling tube, bring back the entire volume of remaining gas into the measuring pipette.
7. Close the connection and measure the volume as before.
8. Repeat Steps No. 5, 6, and 7 until there is no further gas absorbed from contact with the potassium hydroxide solution.

Note.—The apparatus must be free from leaks. Keep the glass stopcocks well greased.

Calculations.

$$\frac{\text{Ml. of gas absorbed} \times 100}{A} = \text{per cent carbon dioxide}$$

Hydrogen, Methane and B.t.u. in Sewage Gas.

Discussion: Sec. III, page 186.

1. Record the volume of gas remaining in the measuring pipette from the carbon-dioxide determination. Let this volume in ml. = B
2. Discard all but 10 ml. of this gas.
3. Lower the leveling tube and open the stopcock to the air, drawing in air until the volume is about 95 to 100 ml.
4. Measure accurately the volume in ml. of the mixture.
5. Allow the gases to mix thoroughly.
6. Close the stopcock and the clamp on the leveling-tube connection and explode or burn the gas in the pipette.
7. Allow the gas to cool to room temperature, open the check on the leveling tube and read the volume in ml. of gas remaining in the pipette.
8. Determine the amount of carbon dioxide produced by passing the gases into the potassium hydroxide pipette several times until no further loss in volume is obtained.
9. Again read the volume of gas in the measuring pipette. Let ml. in Step No. 4 − ml. in Step No. 7 = C. Let ml. in Step No. 7 − ml. in Step No. 9 = D.

Calculations.

$$\frac{10BD}{A} = \text{per cent methane}$$

$$\frac{6.67B(C - 2D)}{A} = \text{per cent hydrogen}$$

(Per cent methane × 10.03) + (per cent hydrogen × 3.29) = B.t.u. per cubic foot (high heat value, 62°F. and 760 mm.)

(Per cent methane × 9.13) + (per cent hydrogen × 2.81) = B.t.u. per cubic foot (low heat value, 62°F. and 760 mm.)

DIVISION III

POLLUTED WATER

Note.—Numbers inclosed in parentheses, thus (3), refer to numbered reagents and solutions in Sec. II.

Dissolved Oxygen, Winkler Method.

See page 38.

Dissolved Oxygen, Rideal-Stewart Modification.

See page 39.

Biochemical Oxygen Demand.

Discussion: Sec. III, page 176.

Case 1.

If the water is not badly polluted, the sample may contain sufficient dissolved oxygen to satisfy the 5-day oxygen demand.

1. Take two samples according to the method given for Sampling for Dissolved Oxygen, page 196. Use 8-oz. glass-stoppered bottles.
2. Make a dissolved-oxygen determination on one sample immediately.
3. Incubate the other sample at 20°C. for 5 days.
4. Make a dissolved-oxygen determination on the incubated sample.

Calculations.

P.p.m. of D.O. before incubation — p.p.m. of D.O. after incubation = p.p.m. 5-day B.O.D.

Case 2.

If the stream is badly polluted, the sample will require dilution prior to incubation.

1. Take two or more samples according to the method given for Sampling for Dissolved Oxygen, page 196.
2. Siphon 750 ml. of diluting water (71) into a liter graduate.
3. Mix the sample and carefully siphon 250 ml. of the sample into the diluting water contained in the graduate.
4. Mix by means of a plunger-type stirring rod, being as careful as possible to prevent aeration. This is a 25 per cent dilution.
5. Fill two 8-oz. bottles with the dilution by means of a siphon, insert the stoppers and place one bottle in the incubator.
6. Now remove by means of the siphon all but 400 ml. of the 25 per cent dilution remaining in the graduate.
7. Add diluting water to the liter mark, mix as before and fill two more bottles with this dilution. This is a 10 per cent dilution.
8. Insert the stoppers and place one bottle in the incubator.
9. Make a dissolved-oxygen determination immediately on the other two bottles.
10. After 5 days make a dissolved-oxygen determination on the incubated samples.

Note.—If an air incubator is to be used, it will be necessary to fit each bottle with a water seal made of heavy rubber tubing, or special B.O.D. bottles may be used which have a seal provided. Seals will not be necessary if the bottles are submerged in a water incubator.

Calculations.

$$\frac{(\text{P.p.m. of D.O. before incubation} - \text{p.p.m. of D.O. after incubation}) \times 100}{\text{Per cent dilution}} = \text{p.p.m. 5-day B.O.D.}$$

Cyanides, Qualitative Test.

Reagents: Sec. II (84), (85).

Discussion: Sec. III, page 186.

1. Place 1 ml. of phenolphthalin (not phenolphthalein) solution (84) and 0.5 ml. of copper sulfate solution (85) in each of two test tubes.
2. To one add 15 ml. of freshly boiled and cooled distilled water.
3. To the other add 15 ml. of the sample.
4. A pink color, which develops immediately, shows the presence of cyanides. This test is sensitive to about 0.4 p.p.m. cyanide (CN).

Note.—On standing, the pink color may develop even in the distilled water due to the oxidation of the phenolphthalin by the dissolved oxygen. The test is not entirely specific for cyanides as chlorine and some other oxidizing agents give the same test.

Combined Acids and Iron in Spent Pickling Liquors and Similar Wastes.

Reagents: Sec. II (16), (86), (95).

Discussion: Sec. III, page 187.

1. Pipette 10 ml. of the waste into a 100-ml. volumetric flask and make up to the mark with distilled water.
2. Mix thoroughly and pipette 10 ml. (or a larger portion depending upon the concentration of the waste) into a 250-ml. beaker and dilute with about 50 ml. of distilled water.
3. Add from a burette a measured quantity of 0.2N sodium hydroxide (86) and record the amount used. The amount used should be sufficient to give a pink color upon the addition of phenolphthalein to the filtrate in Step No. 4.
4. Heat gently for a few minutes and filter. If an excess of sodium hydroxide has been added, the filtrate will give a pink color with a few drops of phenolphthalein.
5. Rinse the beaker and wash the paper and precipitate with small portions of hot water.
6. Add a few drops of phenolphthalein to the filtrate and washings and titrate with 0.1N sulfuric acid (95) until the pink color just disappears. Record the ml. of acid used.

Calculations.

$$\frac{\text{Ml. 0.2N NaOH} - (0.5 \times \text{ml. 0.1N } H_2SO_4) \times 98.1}{\text{Ml. of portion of diluted sample used in Step No. 2}} = \text{gm. per liter of combined acid and iron expressed as } H_2SO_4$$

7. Pipette 10 ml. of the diluted waste from Step No. 1 into a beaker and add about 40 ml. of distilled water and 5 ml. of concentrated hydrochloric acid.
8. Add about 10 ml. of bromine water (16), cover with a watch glass and boil for about 10 min.
9. Make distinctly alkaline to litmus with concentrated ammonium hydroxide and boil gently for 10 min.
10. Ignite, cool and weigh a crucible.

11. Filter the precipitate onto a quantitative filter paper and rinse and wash with hot distilled water.
12. Place the paper in the crucible, ignite, cool in a desiccator and weigh.

Calculations.

$$\frac{\text{Gain in weight (gm.)} \times 19{,}000}{\text{Ml. of portion of diluted sample used in Step No. 2}} = \text{gm. } FeSO_4 \text{ per liter}$$

(Gm. combined acid and iron per liter) − (gm. $FeSO_4$ per liter × 0.645) = gm. H_2SO_4 per liter

Gm. $FeSO_4$ per liter × 0.368 = gm. Fe per liter

Iron, Volumetric Method.

Reagents: Sec. II (94).
Discussion: Sec. III, page 188.

Note.—This method is adapted to the determination of iron in metal pickling liquors, other ferric and ferrous iron solutions and iron coagulants.

1. Use an appropriate portion of the sample depending upon the iron content. The portion should not contain more than 0.15 gram Fe. Usually about 1 ml. of the concentrated iron solutions is sufficient. In order to measure this amount more accurately than is possible with a 1-ml. pipette, pipette 10 ml. of the sample into a 100-ml. volumetric flask, dilute to the mark, mix thoroughly and use a 10-ml. portion.
2. Measure the sample into a casserole and carefully add 4 ml. of concentrated sulfuric acid.
3. Evaporate over a free flame, keeping the casserole in constant motion, until the white fumes of sulfuric acid appear.
4. Cool and carefully add about 50 ml. of distilled water.
5. Rinse the solution into a 250-ml. Erlenmeyer flask, keeping the volume below 100 ml.
6. Add 2 grams of iron-free zinc (granular) and carefully warm until the zinc is dissolved.
7. Titrate while hot with 0.1N potassium permanganate (94) to the first permanent pink color. Record the ml. of permanganate used.

Note.—If iron-free zinc is not available, carry a sample of distilled water through the same procedure. The ml. of permanganate required for the

blank represents the iron in the zinc used and must be subtracted from the ml. of permanganate used for the sample.

Calculations.

$$\frac{\text{Ml. of 0.1N } KMnO_4 \times 5.584}{\text{Ml. of sample used}} = \text{gm. Fe per liter}$$

Gm. Fe per liter $\times$ 2.905 = gm. $FeCl_3$ per liter
Gm. Fe per liter $\times$ 2.72 = $FeSO_4$ per liter

$$\frac{\text{Gm. per ml.} \times 100}{\text{Specific gravity}} = \text{per cent by weight}$$

Alkalies.

Reagents: Sec. II (2), (3), (4), (95).
Discussion: Sec. III, page 189.

1. Measure an appropriate amount of the sample (100 ml. or a smaller amount diluted to 100 ml. with distilled water) into an Erlenmeyer flask.
2. Add 3 drops of phenolphthalein (2).
3. If the solution becomes pink, hydroxide or carbonate alkalinity is present. Add 0.1N sulfuric acid (95) or, if the alkalinity is slight, 0.02N sulfuric acid (3), drop by drop, from a burette until the pink color just disappears. Record the ml. of acid used.
4. Add 3 drops of methyl orange (4) and again titrate with the 0.1N sulfuric acid to the first slight change in color. Record the ml. of acid used.

Calculations.

Let P = ml. 0.1N H_2SO_4 used for the phenolphthalein titration
Let T = the sum of the ml. used for the phenolphthalein and methyl orange titrations

1. When $P = T$

$$\frac{P \times 1.7}{\text{Ml. of sample}} = \text{gm. hydroxide (OH) per liter}$$

2. When P is $> \frac{1}{2}T$

$$\frac{(2P - T) \times 1.7}{\text{Ml. of sample}} = \text{gm. hydroxide (OH) per liter}$$

$$\frac{2(T - P) \times 3.0}{\text{Ml. of sample}} = \text{gm. carbonate } (CO_3) \text{ per liter}$$

3. When $P = \frac{1}{2}T$

$$\frac{T \times 3.0}{\text{Ml. of sample}} = \text{gm. carbonate } (CO_3) \text{ per liter}$$

4. When P is $< \frac{1}{2}T$

$$\frac{2P \times 3.0}{\text{Ml. of sample}} = \text{gm. carbonate } (CO_3) \text{ per liter}$$

$$\frac{(T - 2P) \times 6.1}{\text{Ml. of sample}} = \text{gm. bicarbonate } (HCO_3) \text{ per liter}$$

5. When $P = 0$

$$\frac{T \times 6.1}{\text{Ml. of sample}} = \text{gm. bicarbonate } (HCO_3) \text{ per liter}$$

Note.—If 0.02N H_2SO_4 was used, the results of the above calculations must be divided by 5.0.

Phenols, Baylis Modification of Gibb's Method.

Reagents: Sec. II (55), (87), (88), (98), (100), (101).
Discussion: Sec. III, page 189.

Note.—This method is designed for water, sewage or wastes having a phenol content up to 50 p.p.m. For wastes (ammonia-still liquors, etc.) having a phenol content greater than 50 p.p.m., use the bromine method which follows this procedure.

Case 1.

For sewage or waste containing from 0.05 to 50 p.p.m. phenol.

1. Pipette 200 ml. of the sample into a Kjeldahl flask and acidify with phosphoric acid (98) until just acid to methyl orange. (If the concentration is greater than 0.5 p.p.m., a smaller volume diluted to 200 ml. should be used.)
2. Connect the flask to the condenser of an ammonia still and distill until about 180 ml. of distillate is collected in a 200-ml. volumetric flask.
3. Add 20 ml. of distilled water to the residue in the flask and continue the distillation until 200 ml. total distillate is collected.
4. If necessary, filter through asbestos to remove fats.
5. Determine the ml. of buffer solution (88) necessary to adjust the distillate to pH 9.6 as follows: Place 50 ml. of the distillate

in a Nessler tube and add 0.5 ml. of buffer solution (88). Test a 2-ml. portion of this solution for pH by adding 3 drops of thymolphthalein (87) or oleo red and comparing the color with pH standards. If pH is too low, add more buffer solution to the 50 ml. of distillate, 0.1 ml. at a time, until a pH of 9.6 is obtained. Record the ml. of buffer solution required.

6. Make up aliquot portions of 1, 2, 5, 25 and 100 ml. of the distillate to 100 ml. in a series of Nessler tubes and add 1 ml. of copper sulfate (100) to each tube.
7. Add a volume of buffer solution (88) to each tube as determined by the following formula:

D = ml. aliquot portion of distillate used
B = ml. of buffer solution required for 50-ml. distillate
V = ml. of buffer required to produce pH of 9.6 in the diluted aliquot portion in each tube

$$V = \left(\frac{D}{50}\right) \times B + \left(\frac{100 - D}{100}\right)$$

8. At this point make up standards by placing 0.1, 0.3, 0.6, 1.0, 1.5, 2.0, 3.0 and 4.0 ml. of standard phenol solution (89) into 100-ml. Nessler tubes. Add 1 ml. of copper sulfate (100) and 1 ml. of buffer solution (88) and make up to the mark with distilled water.
9. Add 1.5 ml. of 2,6-dibromoquinonechloroimide (90) to each standard and to the diluted aliquot distillates. Invert the tubes three times to mix.
10. After standing at least 2 hr., compare the colors and select the standard and aliquot having similar colors.

Calculations.

$$\frac{\text{Ml. of standard phenol} \times 2{,}000}{\text{Ml. aliquot portion} \times \text{ml. sample}} = \text{p.p.m. phenol}$$

Case 2.

For polluted waters or water containing minute amounts of phenol.

1. Measure 800 ml. of the sample into a liter Kjeldahl flask, add 4 ml. of 12N sodium hydroxide (55) and boil down to about 200 ml.

2. Proceed according to Steps No. 1 to 6, inclusive, in Case 1 except that in Step No. 5 buffer solution (101) should be used in place of (88).
3. Add a volume of buffer solution (101) to each tube as determined by the following formula:
   D = ml. aliquot portion of distillate used
   B = ml. of buffer required for 50 ml. of distillate
   V = ml. of buffer required to produce pH of 9.6 in the diluted aliquot portion in each tube

$$V = \left(\frac{D}{50}\right) \times B + 5\left(\frac{100 - D}{100}\right)$$

4. Proceed according to Steps No. 8 to 10 inclusive in Case 1 except that 5 ml. of buffer (101) is used in Step No. 8 in place of (88).

Calculations.

$$\frac{\text{Ml. of standard phenol} \times 2{,}000}{\text{Ml. of aliquot portion} \times \text{ml. of sample}} = \text{p.p.m. phenol}$$

Phenol, Bromine Method.

Reagents: Sec. II (60), (91), (92), (93).
Discussion: Sec. III, page 189.

Note.—This method is designed for wastes having phenol contents above 50 p.p.m.

1. Place 100 ml. of the sample (a smaller quantity diluted to 100 ml. may be used if the phenol content is very high) into a Kjeldahl flask.
2. Add 5 ml. of 20 per cent sodium hydroxide (91) and boil off the ammonia or until the vapors have no alkaline effect on moist red litmus paper.
3. Add 1 gram of lead carbonate and boil 1 min. more.
4. Add 15 grams of sodium bicarbonate and make up to about 100 ml.
5. Add boiling chips to prevent bumping and a drop of castor oil to prevent foaming.
6. Connect to the ammonia still and distill until about 90 ml. of distillate has been collected in an Erlenmeyer flask.

7. Cool the distillate and add 5 ml. of concentrated hydrochloric acid.
8. Add 0.1N bromine solution (93) slowly from a burette, with constant mixing, until a yellow color indicates an excess of bromine. Record the ml. of 0.1N bromine used. (If the yellow color disappears, add more bromine.)
9. Stopper and allow to stand in cold water for 15 min.
10. Add about 2 grams of potassium iodide crystals.
11. Titrate with 0.1N sodium thiosulfate (92) using starch (60) as an indicator near the end of the titration. Record ml. of thiosulfate used.

Calculations.

$$\frac{(\text{Ml. 0.1N bromine} - \text{ml. of 0.1N sodium thiosulfate}) \times 1{,}567}{\text{Ml. of sample used}} = \text{p.p.m. phenol}$$

DIVISION IV

BOILER WATERS

FIELD AND PLANT TESTS

The following tests are designed for plant and field use where a minimum of equipment and laboratory-trained personnel is available. It is common practice, particularly in many of the smaller plants, to use a special measuring device for water samples. This device may be a graduated cylinder, volumetric flask or pipette graduated to deliver 58.3 ml. of sample. The graduated cylinder is often further marked to deliver 29.15 ml. and 14.57 ml. These are ½ and ¼ portions, respectively, of the total volume of the cylinder. The use of the odd quantities allows the operator to convert his readings of standard-solution volumes direct to grains per gallon of the constituent for which the test is made. The directions which follow are designed to maintain this practice. Alternate procedures are given which allow the use of the volume of sample usually employed in water laboratories. This change, however, necessitates the use of standard solutions of different normalities than commonly used in the field or boiler plant. Directions are given for preparing solutions of the required normality. The same results may be obtained by changing the factors in the calculations. Such changes may not be desirable in many cases where untrained personnel is employed.

Alkalinity.

Reagents: Sec. II (2), (3), (121), alternate (116).
Discussion: Sec. III, page 140.

1. If the sample contains suspended material, filter somewhat more than the amount required through a filter paper.
2. Measure 58.3 ml. of the filtered sample into an Erlenmeyer flask or white porcelain evaporating dish.
3. Place the same quantity of distilled water into a second flask or dish.

4. Add 3 drops of phenolphthalein indicator (2) to each.
5. If the sample becomes pink, add 0.02N sulfuric acid (3) from a burette until the pink color just disappears. Record the number of ml. used as P.
6. Add 2 drops of xylene cyanole-methyl orange indicator (121) to each.
7. If the sample becomes yellow, continue to add the acid until the first change in color is noted when compared with the distilled water sample. The end point is purple. Record the total number of ml. of acid used for both the phenolphthalein and xylene cyanole-methyl orange titration as T.

Note.—A 100-ml. sample may be used as an alternate procedure. In this case the titration is made with 0.03424N sulfuric acid (116). This does not affect the remainder of the procedure nor the calculations.

Calculations.

T = total alkalinity in grains per gallon (g.p.g.)
P = phenolphthalein alkalinity in g.p.g.
If $P = T$, hydroxide alkalinity (g.p.g.) = P
If $P > \frac{1}{2}T$, hydroxide alkalinity (g.p.g.) = $2P - T$
and normal carbonate alkalinity (g.p.g.) = $2T - P$
If $P = \frac{1}{2}T$, normal carbonate alkalinity (g.p.g.) = T
If $P < \frac{1}{2}T$, normal carbonate alkalinity (g.p.g.) = $2P$
and bicarbonate alkalinity (g.p.g.) = $T - 2P$
If $P = 0$, bicarbonate alkalinity (g.p.g.) = T

All results are expressed in terms of $CaCO_3$. The results are sufficiently accurate for most control purposes unless the water is highly colored with decomposed organic matter.

Alkalinity, Barium Chloride Method.

(Use only for waters containing hydroxide alkalinity.)

Reagents: Sec. II (2), (3), (22), alternate (116).

1. Follow Step Nos. 1 to 5 inclusive in preceding test. Record the number of ml. of acid used as P.
2. Measure a second 58.3-ml. portion into a small beaker. (The alternate volume of sample and normality of acid suggested in Note in preceding test may be used without changing the procedure or calculations.)
3. Add 10 ml. of barium chloride (22).

4. Filter the precipitated material onto a filter paper and collect the filtrate in a flask or dish. (Filtration is not necessary but is desirable.)
5. Add 3 drops of phenolphthalein (2) and titrate with the standard sulfuric acid (3) until the pink color just disappears. Disregard any return of this color. Record the number of ml. of acid used as H.

Calculations.

Hydroxide as OH (g.p.g.) = $0.34H$
Hydroxide alkalinity (g.p.g.) as $CaCO_3$ = H
Total carbonate alkalinity (g.p.g.) as $CaCO_3$ = $(P - H)2$
Total alkalinity (g.p.g.) as $CaCO_3$ = $(P - H)2 + H$
To convert alkalinity as $CaCO_3$ to g.p.g. of soda ash, multiply by 1.06.

Acidity.

Reagents: Sec. II (117), (121), alternate (118).
Discussion: Sec. III, page 143.

1. Measure 58.3 ml. of the water into an Erlenmeyer flask or white porcelain evaporating dish. (See Note.)
2. Add 3 drops of xylene cyanole-methyl orange indicator (121).
3. If the solution is purple, add 0.02N sodium carbonate (117) from a burette until the first change of color. The end point is green. Record the number of ml. of sodium carbonate used.

Note.—A 100-ml. sample may be used if the titration is made with 0.03424N sodium carbonate (118). The calculations are not affected.

Calculations.

Number of ml. of sodium carbonate used = g.p.g. acidity in terms of $CaCO_3$

Hardness, B and B Soap Method.

Reagents: Sec. II (7A).
Discussion: Sec. III, page 152.

1. By means of a graduated cylinder, measure 40 ml. of the sample into a 4-oz. glass bottle.
2. Add the B and B soap solution (7A) a drop at a time from a calibrated dropping bottle prepared as described under (7A). Count the drops as they are added.
3. Shake the bottle vigorously between the additions of the soap solution.

4. Continue the addition of the soap solution until a lather remains unbroken for 5 min. over the entire surface of the water while the bottle lies on its side. The quantity added between each interval will depend upon the hardness of the water. If the water is soft, the mixture should be shaken between each drop. (In order to avoid mistaking the false magnesium end point for the true one, record the number of drops used on the first appearance of a lather and then continue the addition of soap solution until the true end point is definitely reached.)
5. Record the total number of drops of soap solution required.

Calculations.

$$\frac{(\text{Drops of B and B solution required} - 2) \times 5.5}{\text{Drops to make 1 ml.}} = \text{g.p.g. hardness as } CaCO_3$$

Note.—This method for the determination of soap hardness is of sufficient accuracy for the usual plant or field tests. It has an accuracy between 5 and 10 per cent. The method is especially adapted for use with zeolite-softened water. Zero-hardness water should not require more than 4 drops to produce a lather.

Sulfates, Benzidine Method.

Reagents: Sec. II (2), (119), (120), alternate (122).
Discussion: Sec. III, page 159.

1. If the sample contains suspended matter, filter about 70 ml. through a filter paper.
2. Measure 58.3 ml. of the filtered sample into a 250-ml. Erlenmeyer flask.
3. Add 10 ml. of benzidine hydrochloride solution (119) and mix by giving the flask a whirling motion.
4. Allow the mixture to stand for about 10 min.
5. Filter the precipitated benzidine sulfate onto a small filter paper. The solution should be refiltered through the same paper until the filtrate is clear.
6. Add 1 ml. of benzidine hydrochloride solution to the filtrate. If further precipitation takes place, filter through the same paper. Repeat the addition of benzidine sulfate until all of the sulfate is precipitated and removed to the paper.

7. Wash the flask and precipitate on the paper with several small portions of distilled water. Allow each portion to drain through the paper before the next is added.
8. Transfer the paper containing the benzidine sulfate to the original flask, add about 25 ml. of distilled water and 2 drops of phenolphthalein indicator (2).
9. Add 0.143N (N/7) sodium hydroxide (120) from a burette until the first permanent pink color is obtained. Be sure that the paper is completely disintegrated and that the color is permanent.
10. Record the ml. of standard sodium hydroxide used.

Note.—A 50-ml. sample may be used, in which case the normality of the sodium hydroxide should be 0.121(122). Calculations are the same.

Calculations.

Ml. of standard NaOH × 10 = g.p.g. SO_4 as sodium sulfate (Na_2SO_4)
Ml. of standard NaOH × 6.32 = g.p.g. SO_4

Chlorides.

Reagents: Sec. II (2), (3), (4), (13), (117), (123).
Discussion: Sec. III, page 163.

1. Filter, if needed, a portion of the sample to remove suspended matter.
2. Measure 58.3 ml. of the filtered sample into a porcelain evaporating dish.
3. Add 3 drops of phenolphthalein indicator (2) and, if a pink color develops, add 0.02N sulfuric acid (3) until the pink color just disappears. Do not record the amount of acid used.
4. If the pink color is not obtained with phenolphthalein, add 3 drops of methyl orange (4).
5. If the solution becomes red, add 0.02N sodium carbonate (117) until the color changes to orange. If the solution is yellow upon the addition of methyl orange, omit the sodium carbonate. Do not record the amount used.
6. Add 1 ml. of potassium chromate indicator (13).
7. Add 0.0172N (N/58.3) silver nitrate (123) from a burette, a few drops at a time, with constant stirring until the first permanent reddish color is obtained. This color change may be more readily determined by comparison with a distilled-water

sample to which 1 ml. of potassium chromate indicator has been added. Record the ml. of silver nitrate used.

8. If more than 10 ml. of silver nitrate is required, repeat the entire procedure using ½ or ¼ samples diluted to approximately 58.3 ml. with distilled water.

Calculations.

Ml. 0.0172N $AgNO_3$ = g.p.g. chlorides as NaCl
G.p.g. chlorides as NaCl × 0.608 = chlorides as Cl
Note.—See page 15 for chloride determination used in water laboratory.

Free Carbon Dioxide.

Reagents: Sec. II (2), (4), (117).
Discussion: Sec. III, page 143.

Note.—This test must be made on a sample of the water immediately after collection.

1. Measure 58.3 ml. of the sample into a white porcelain evaporating dish.
2. Add 6 drops of phenolphthalein indicator (2).
3. If a pink color develops, no carbon dioxide is present.
4. If the solution is not pink, add 0.02N sodium carbonate (117) from a burette with constant stirring until the pink color which develops remains throughout the solution for a period of 5 sec. The pink color may disappear due to absorption of carbon dioxide from the air.
5. Record the ml. of 0.02N sodium carbonate used.

Calculations.

Ml. 0.02N Na_2CO_3 = g.p.g. CO_2 as $CaCO_3$
G.p.g. CO_2 as $CaCO_3$ × 0.44 = g.p.g. CO_2
Note.—This test is invalid in the presence of mineral acids. To test for mineral acids add methyl orange (4) to a small portion of the sample. If the color is yellow, mineral acids are not present. If it is red, mineral acids are present. (See Note under Acidity, page 92.)

Dissolved Solids.

The gravimetric test for dissolved solids (see page 5) usually cannot be made in the field or plant. Various types of electrical equipment and hydrometers are available for rough estimates of the content of mineral salts dissolved in a water. Most of these

types produce results of sufficient accuracy for plant purposes. Specific instructions for the operation of the equipment are furnished by the manufacturer.

Sulfates.

Some plants find it convenient to use special types of turbidimeters (for example, the Parr turbidimeter) for the determination of sulfates in a water sample. These instruments measure the turbidity produced by the precipitation of the sulfates as barium sulfate. They are standardized against solutions of known sulfate content. Specific instructions for their operation are furnished by the manufacturer.

Hydrogen-ion Concentration (pH).

See page 10.

Qualitative Tests.

Reagents: Sec. II (123), (124).

1. *Chlorides and Carbonates*—Place about 10 ml. of the sample in a test tube and add about 1 ml. of silver nitrate (123). A white precipitate indicates chlorides and carbonates. Add a few drops of concentrated nitric acid. The carbonates will dissolve, leaving a precipitate of silver chloride.
2. *Bicarbonates*—Place about 10 ml. of the sample in a test tube and boil. If a white precipitate appears, bicarbonates of calcium and/or magnesium are indicated.
3. *Sulfates*—Filter the sample from Step No. 2, add 1 ml. of sodium carbonate solution (124) to the filtrate, and heat. A white precipitate indicates the sulfates of calcium and possibly magnesium.
4. *Calcium sulfate*—Place about 5 ml. of the sample in a test tube and add 5 ml. of 95 per cent alcohol. A white precipitate indicates calcium sulfate.

LABORATORY TESTS

Note.—The following additional tests for boiler waters are designed for the laboratory with complete equipment and trained personnel. Whenever possible, reference is made to procedures given in other portions of the manual so as to avoid repetition.

Total Solids.

Use the procedure given on page 3.

Dissolved Solids.

Use the procedure given on page 6.

Alkalinity.

Use the procedure given on page 90.

Hydroxide.

Use the procedure on page 91.

Dissolved Oxygen.

Use the Rideal-Stewart modification of the Winkler method given on page 39.

Silica, Colorimetric Method.

Reagents: Sec. II (77), (125), (126), (127), (128).
Discussion: Sec. III, page 165.

Note.—This method is required for water containing phosphates.

1. Place exactly 110 ml. of the sample into a 350-ml. beaker.
2. Add 50 ml. of borate mixture (125) and 2 ml. of calcium chloride solution (126).
3. Stir vigorously and allow the mixture to stand for at least 1 hr.
4. Filter to remove the precipitate.
5. Pipette 50 ml. of the filtrate into a 100-ml. Nessler tube and fill to the mark with distilled water.
6. Add 2 ml. of ammonium molybdate solution (77) and 1 ml. of hydrochloric acid solution (127).
7. Allow to stand for 10 min. and compare the color with the permanent color standards (128).

Calculations.

Read the p.p.m. silica direct from color standard table last column (128), page 128.

Total Iron, Colorimetric Method.

Use the procedure given on page 27.

Total Phosphate, Gravimetric Method.

Reagents: Sec. II (2), (77), (78), (79).

1. Fill a 500-ml. volumetric flask to the mark with the water, add about 10 ml. of concentrated nitric acid and mix.
2. Evaporate the sample nearly to dryness in an evaporating dish on a water bath.
3. Add about 50 ml. of distilled water and filter. (This filtration may be omitted if solids are completely dissolved.)
4. Add concentrated ammonium hydroxide until the precipitate which first forms dissolves but slowly on stirring. If too much ammonium hydroxide is added, a permanent precipitate is obtained. In this case carefully add concentrated nitric acid until this precipitate just dissolves.
5. Add 1 or 2 grams of solid ammonium nitrate and heat to 55 to 60°C. on the water bath.
6. Add 20 to 25 ml. of freshly filtered molybdate solution (77) and allow the mixture to remain on the water bath with constant stirring for 30 min.
7. Decant the solution through a filter paper and wash the precipitate in the beaker twice with about 25-ml. portions of distilled water, filtering each washing through the same paper.
8. Transfer the entire precipitate to the paper and wash with portions of distilled water until a portion of the filtrate gives a pink color upon the addition of phenolphthalein indicator (2) and one drop of sodium hydroxide (78).
9. Transfer the precipitate and filter paper to a beaker and dissolve the precipitate in a small excess of 0.3238N sodium hydroxide (78). Record the ml. of hydroxide used.
10. Add a few drops of phenolphthalein indicator (2) and titrate the excess hydroxide with 0.3238N hydrochloric acid (79). Record the ml. of acid used.

Calculations.

$$\frac{(\text{Ml. } 0.3238\text{N NaOH} - \text{ml. } 0.3238\text{N HCl}) \times 58.4}{\text{Volume of sample used}} = \text{g.p.g. } P_2O_5$$

Magnesium.

Use the procedure given on page 20.

Hydrogen-ion Concentration (pH).

Use the procedure given on page 10.

Calcium.

Use the procedure given on page 19.

Sulfite.

Reagents: Sec. II (59), (60), (69).

1. Place 10 ml. of 0.025N iodine (69) and 5 ml. of glacial acetic acid into each of two 250-ml. Erlenmeyer flasks.
2. Add 100 ml. of the freshly collected and cooled, but unfiltered, sample slowly and with constant mixing to one flask, and 100 ml. of distilled water to the other.
3. To the flask containing the sample add from a burette 0.025N sodium thiosulfate (59) until the color of the iodine almost disappears. Add 1 ml. of starch indicator (60) and continue the addition of thiosulfate until the blue color just disappears. Record the ml. of thiosulfate used.
4. Titrate the solution containing the distilled water in the same manner. Record the ml. of thiosulfate used.

Calculations.

Let D = ml. of thiosulfate used for distilled water.
Let S = ml. of thiosulfate used for sample.
$(D - S) \times 0.91$ = g.p.g. sodium sulfite (Na_2SO_3)

Oil.

Reagents: Sec. II (4), (76).

1. Evaporate 1 to 2 liters of the sample to about 50 ml. in an evaporating dish.
2. Scrape the solids from the sides of the dish into the unevaporated portion.
3. Add 2 drops of methyl orange (4) and add hydrochloric acid (76) until the solution is red.
4. Evaporate to dryness and dry in a 103°C. oven.
5. Add 50 ml. of oil-free petroleum ether, warm and stir the solids into the ether to insure complete contact.
6. Weigh a Soxhlett flask.

7. Decant the ether into the flask, filtering through a dry filter paper if necessary.
8. Repeat the extraction twice, using 25-ml. portions of ether.
9. Transfer all of the ether to the flask and evaporate over a warm-water bath from which the flame has been removed.
10. Dry the flask at 103°C., cool in the desiccator and weigh.

Calculations.

$$\frac{\text{Increase in weight (gm.)} \times 58,400}{\text{Volume of sample (ml.)}} = \text{g.p.g. oil}$$

SECTION II

PREPARATION OF REAGENTS AND STANDARD SOLUTIONS

DIVISION I

STOCK STANDARD SOLUTIONS

A. Sulfuric Acid.

Discussion: Sec. III, page 136.

a. Place a few grams of pure anhydrous sodium carbonate in a small crucible and heat on a wire gauze over a small Bunsen flame to drive off the moisture. (Drying in a 103°C. oven is preferred, if such an oven is available.)

b. Cool in a desiccator and transfer the carbonate to a clean dry weighing bottle. Handle the weighing bottle with a clean dry cloth or piece of paper.

c. Weigh the bottle and contents.

d. Carefully transfer about 0.2 gram of the carbonate to a 250-ml. Erlenmeyer flask and reweigh the bottle and contents. The difference in weight is the weight of the carbonate used.

e. Weigh out a second portion into another flask in the same manner.

f. Dissolve each sample in about 80 ml. of distilled water and add 3 drops of methyl orange (4).

g. Measure by means of a small graduate about 6 ml. of concentrated sulfuric acid and transfer this and several rinsings of the graduate to a liter bottle. Dilute to approximately 1 liter with distilled water and mix.

h. Rinse a clean burette several times with about 10-ml. portions of the acid solution and then fill completely.

i. Add the acid solution from the burette to the carbonate solution in each flask with constant mixing until the first change in color. Select as the end point the faintest trace

of red in the yellow solution giving a slight orange tint. Always titrate to the same color. Record the ml. of acid used in each case.

Calculations.

$$\frac{\text{Weight of } Na_2CO_3 \text{ (gm.)} \times 1{,}000}{\text{(Ml. of acid used in Step } i\text{)} \times 53} = \text{normality of acid}$$

The solution should be approximately 0.2N.

Example.

Sample number	Weight of weighing bottle and contents, gm.		Weight of sodium carbonate used, gm.	Titration results	
	Before removing sample	After removing sample		Ml. of H_2SO_4 used	Normality of acid solution
1	20.6971	20.2833	0.4138	37.1	0.2105
2	20.2833	19.8990	0.3843	34.5	0.2102

The values in the last column were obtained as follows:

Sample No. 1:

$$\frac{0.4138 \times 1{,}000}{37.1 \times 53} = 0.2105$$

Sample No. 2:

$$\frac{0.3843 \times 1000}{34.5 \times 53} = 0.2102$$

B. Sodium Hydroxide.

Discussion: Sec. III, page 138.

a. Weigh in a beaker on a trip scale about 30 grams of pure sodium hydroxide sticks.

b. Add about 100 ml. of distilled water and let stand until the surface coating of the sticks has dissolved. (This removes any carbonate coating.)

c. Pour off the supernatant liquor and dissolve the remaining sodium hydroxide (there should be about 20 grams remaining) in freshly boiled and cooled distilled water.

d. Transfer the solution to a liter bottle, dilute to approximately 1 liter with boiled distilled water and mix thoroughly.

e. Fill a clean burette with the standard sulfuric acid stock solution (*A*).

f. Pipette two portions of exactly 10 ml. each of the sodium hydroxide solution into two 250-ml. Erlenmeyer flasks, dilute to about 100 ml. and add 3 drops of methyl orange (4).

g. Titrate with the acid solution from the burette until the first permanent change in color is obtained. Select as the end point the faintest trace of red in the yellow solution giving a slight orange tint. Always titrate to the same color. Record the ml. of acid used.

Calculations.

$$\frac{\text{Ml. of sulfuric acid} \times \text{normality of sulfuric acid}}{\text{Ml. of sodium hydroxide}} = \text{normality of sodium hydroxide}$$

The solution should be approximately 0.5N.

C. Hydrochloric Acid.

Discussion: Sec. III, page 137.

a. Place approximately 93 ml. of concentrated hydrochloric acid in a liter bottle, make up to approximately 1 liter with distilled water and mix thoroughly.

b. Standardize the solution against sodium carbonate in the manner described for the standardization of sulfuric acid (*A*) except use about 1.0 gram of sodium carbonate in Step (*d*) and omit Step (*g*).

Note.—It is possible to standardize the hydrochloric acid against the sodium hydroxide (*B*) by titration. A direct standardization, however, is preferred.

Calculations.

$$\frac{\text{Wt. of sodium carbonate (gm.)} \times 1{,}000}{\text{Ml. of acid used} \times 53} = \text{normality of acid}$$

The solution should be approximately 1N.

D. Sodium Thiosulfate.

Discussion: Sec. III, page 139.

a. Dissolve exactly 24.820 grams of sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) in distilled water and make up to 1 liter.

b. Add 1.5 grams of ammonium carbonate and 5 ml. of chloroform as a preservative. (This solution may also be preserved by adding 0.2 per cent by weight of sodium hydroxide.)

c. This solution is 0.1N and is considered sufficiently accurate to be diluted for use in the ordinary dissolved-oxygen determinations. As it does not keep well it should be made up new after a few weeks or restandardized. For restandardization or a more accurate standardization of the original solution continue with steps (*d*) to (*g*).

d. Weigh exactly 3.250 grams of potassium biniodate ($KIO_3 \cdot HIO_3$), dissolve in distilled water, make up to 1 liter and mix thoroughly.

e. Dissolve about 5 grams of potassium iodide crystals in about 100 ml. of distilled water in a 250-ml. Erlenmeyer flask.

f. Add 10 ml. of dilute sulfuric acid (1 to 10) and exactly 25 ml. of the biniodate solution.

g. Titrate with the sodium thiosulfate solution using starch (60) as an indicator near the end of the titration (when a pale straw color is obtained). Record the ml. of thiosulfate used.

Calculations.

$$\frac{\text{Ml. of biniodate solution} \times 0.1}{\text{Ml. of thiosulfate solution}} = \text{normality of sodium thiosulfate}$$

E. Potassium Permanganate.

Discussion: Sec. III, page 140.

a. Dissolve exactly 13.400 grams of C.P. sodium oxalate ($Na_2C_2O_4$) or 14.210 grams of C.P. ammonium oxalate crystals [$(NH_4)_2C_2O_4 \cdot H_2O$] in distilled water and make up to 1 liter. This solution is 0.2N.

b. Dissolve about 6.4 grams of potassium permanganate in distilled water, make up to 1 liter and mix thoroughly. Standardize as follows:

c. Pipette 25 ml. of the sodium oxalate solution into a 250-ml. Erlenmeyer flask and dilute to about 100 ml. with distilled water.

d. Add 10 ml. of 1 to 3 sulfuric acid (63) and heat to 90°C.

e. Titrate while hot with the potassium permanganate solution to the first permanent pink color. Record the ml. of permanganate used.

Calculations.

$$\frac{\text{Ml. of sodium oxalate} \times 0.2}{\text{Ml. of potassium permanganate}} = \text{normality of potassium permanganate}$$

DIVISION II

REAGENTS AND STANDARD SOLUTIONS

Note.—Letters in parentheses, thus (*A*), refer to Stock Standard Solutions, Sec. II, Div. I. Most of the following instructions are for making liter quantities of solutions. Frequently 50 to 500 ml. should be sufficient, especially where students are making solutions for individual use, in which case proportional quantities of chemicals are used.

1. **Asbestos Fiber Emulsion.**—Place about 2.5 grams of finely shredded, acid-washed asbestos fiber in a liter bottle. Add 900 ml. of distilled water and shake thoroughly.
2. **Phenolphthalein Indicator.**—Dissolve about 5.0 grams of phenolphthalein in 1 liter of 50 per cent alcohol. Neutralize the solution with 0.02N sodium hydroxide (5). (To make 1 liter of 50 per cent alcohol, dilute 526 ml. of 95 per cent grain alcohol to 1 liter with boiled distilled water.)
3. **Standard 0.02N Sulfuric Acid.**—Dilute the exact quantity of stock standard sulfuric acid solution (*A*) as determined by the following formula to 1 liter with distilled water.

$$\frac{20}{\text{Normality of stock } H_2SO_4} = \text{ml. stock } H_2SO_4 \text{ necessary to produce 1 liter of 0.02N } H_2SO_4$$

4. **Methyl Orange Indicator.**—Dissolve about 0.5 gram of methyl orange in 1 liter of distilled water.
5. **Standard 0.02N Sodium Hydroxide.**—Dilute to 1 liter with boiled distilled water the exact quantity of stock standard sodium hydroxide (*B*) as determined by the following formula:

$$\frac{20}{\text{Normality of stock NaOH}} = \text{ml. stock NaOH necessary to produce 1 liter of 0.02N NaOH}$$

6. Standard N/44 Sodium Hydroxide.—Dilute to 1 liter with boiled distilled water the exact quantity of stock standard sodium hydroxide (*B*) as determined by the following formula:

$$\frac{22.73}{\text{Normality of stock NaOH}} = \text{ml. of stock NaOH necessary to produce 1 liter of } \frac{N}{44} \text{ NaOH}$$

7. Standard Soap Solution.

a. Make up a stock soap solution by shaking 80 to 100 grams of pure powdered castile soap (or shavings obtained by scraping a bar of castile soap) with 1 liter of 80 per cent grain alcohol (25), let stand overnight and decant. This stock solution will be seven to ten times as strong as the standard soap solution. (If few soap-hardness tests are made it may be more satisfactory to omit the stock solution. In that case make up a solution of approximate standard strength with about 12 grams of soap to 1 liter of 80 per cent alcohol. Adjust this solution in the same manner as given below for the stock solution.)

b. Prepare a standard calcium solution by dissolving exactly 0.5000 gram of pure calcium carbonate in about 5 ml. of 1 to 3 hydrochloric acid. Add about 40 ml. of boiled and cooled distilled water and add ammonium hydroxide until slightly alkaline to litmus. Make up to exactly 500 ml. with freshly boiled and cooled distilled water. 1 ml. = 1 mg. $CaCO_3$. (Hardness = 1,000 p.p.m.)

c. Determine the lather factor by adding the stock soap solution, drop by drop, from a burette to a 50-ml. portion of freshly boiled and cooled distilled water contained in a 4- to 8 oz. glass bottle, shaking between the additions. When sufficient soap solution has been added to produce a lather which remains over the entire surface of the water for 5 min. (bottle should be laid on its side), record the ml. of solution added. This is the lather factor = *L.F.*

d. Pipette 25 ml. of the standard calcium solution into a bottle, add 25 ml. of freshly boiled and cooled distilled water and titrate with the stock soap solution as above until a perma-

nent lather is obtained, and let ml. of the stock solution used = K.

c. Make a standard soap solution so that 1 ml. equals 1 mg. of $CaCO_3$. The amount of stock soap solution required to make 1 liter of standard soap solution may be obtained by using the following formula:

$$K \text{ (from Step } d) - L.F. \text{ (from Step } c) \times 40 = \text{ml. stock soap solution required}$$

Dilute this quantity of stock solution to 1 liter with 80 per cent alcohol (25).

f. Determine the lather factor for this standard soap solution in the same manner as described in above Step (*c*) for stock soap solution. Record the value on the bottle.

Note.—The above prepared standard soap solution will give results sufficiently accurate for ordinary purposes if hardness of the sample is computed as in the formula under Calculations in the Soap Test for Hardness. A more accurate standardization of the soap solution may be obtained as follows: Prepare waters containing known amounts of hardness. The standard calcium solution, of Step (*b*) above, may be used to prepare waters of 40, 60, 80, 100, 120 and 140 p.p.m. hardness by using 2, 3, 4, 5, 6 and 7 ml., respectively, diluted to 50 ml. with freshly boiled and cooled distilled water. The ml. of the standard soap solution required to produce lathers in each of these waters is determined and the results tabulated or a curve plotted. (If an unusually large per cent of the hardness is due to magnesium, it is advisable to make up the set of hard waters from a mixture of the standard calcium solution and a similar magnesium solution.) Then this table or curve is used directly to obtain the hardness of the sample as explained under Calculations in the Soap Test for Hardness.

7A. B and B (Boutron and Boudet) Soap Solution.*

a. Prepare a soap solution by dissolving 40 grams of pure powdered castile soap (or shavings by scraping a bar of castile soap) in about 650 ml. of 95 per cent alcohol and dilute to 1 liter with distilled water. [May use 775 ml. of 80 per cent alcohol (25) instead of the 650 ml. of 95 per cent alcohol.]

* Buswell, A. M., Boutron and Boudet Soap Solution, *Am. Water Works Assoc. Journal*, vol. 9, page 892, 1922.

"Preparation of Boutron and Boudet Soap Solution," The Permutit Co.

b. Place exactly 9 ml. of the standard calcium solution (from Step No. 7*b*, above) in a 3- to 4-oz. bottle, dilute to about 40 ml. with distilled water and titrate with the soap solution, recording the ml. used to produce a lather.

c. Adjust the soap solution with distilled water until 2.4 ml. of soap solution will give a lather with 9 ml. of the standard calcium solution diluted to about 40 ml. with distilled water. Then 1 ml. of this soap solution = 3.77 mg. of $CaCO_3$, which is the required strength of B and B soap solution.

d. Calibrate the dropping bottle to be used in making tests for hardness with the B and B soap solution by counting the drops to deliver 1 ml.

8. **Soda Reagent.**—Dissolve about 2 grams sodium hydroxide and about 2.65 grams of anydrous sodium carbonate in distilled water and make up to 1 liter.

9. **Limewater.**—Add about 3 grams of pure $Ca(OH)_2$ to about 1 liter of distilled water. Shake thoroughly for some time and allow to stand until clear. Use the clear supernatant liquor.

10. **Hydroxylamine Hydrochloride.**—Dissolve about 10 grams of hydroxylamine hydrochloride in 1 liter of distilled water.

11. **Benzidine Hydrochloride.**—Place about 8 grams of benzidine in an agate mortar and add enough water to make a paste. Wash the paste into a liter flask, add about 10 ml. of concentrated hydrochloric acid and make up to the mark. Filter if necessary.

12. **Standard 0.05N Sodium Hydroxide.**—Dilute to 1 liter with boiled distilled water the exact quantity of stock standard sodium hydroxide (*B*) as determined by the following formula:

$$\frac{50}{\text{Normality of stock NaOH}} = \text{ml. stock NaOH necessary to produce 1 liter of 0.05N NaOH}$$

13. **Potassium Chromate Indicator.**—Dissolve about 50 grams of neutral potassium chromate in a small quantity of distilled water. Add silver nitrate solution (14) to

produce a slight red precipitate. Allow to stand overnight and filter. Make up to 1 liter.

14. **Standard Silver Nitrate.**—Dissolve 2.400 grams of C.P. silver nitrate crystals in 1 liter of distilled water. This solution may be standardized against sodium chloride as follows: Dissolve 16.480 grams of C.P. sodium chloride in distilled water and make up to 1 liter in a volumetric flask. Mix well and pipette 100 ml. of this stock solution into a second flask. Make up to 1 liter and mix. Pipette 25 ml. of this solution into a porcelain dish and add 25 ml. of distilled water. Place 50 ml. of distilled water in another dish as a control. Add 1 ml. of potassium chromate indicator to each. Add the silver nitrate solution from a burette drop by drop until the first permanent reddish-brown color remains after stirring. Record the ml. of silver nitrate solution used.

    The number of ml. of silver nitrate solution must be corrected for error due to variation in volume by subtracting $(0.003V + 0.02)$ where V = ml. of liquid at the end of the titration. Adjust the solution so that 2 ml. of the silver nitrate will be exactly equivalent to 1 ml. of the sodium chloride solution. One milliliter of the salt solution contains 1 mg. of chloride radical. Then 1.0 ml. of the silver nitrate solution will be equivalent to 0.500 mg. of the chloride radical.

15. **Aluminum Hydroxide.**—Dissolve 125 grams of pure aluminum sulfate in 1 liter of water. Add ammonium hydroxide until the precipitation is complete. Allow to settle and pour off the supernatant liquor. Wash the precipitate several times by adding distilled water, stirring and decanting to remove chlorides, nitrites and ammonia.

16. **Bromine Water.**—Fill a liter bottle almost full of distilled water. In the hood, carefully pour about 10 ml. of bromine into the bottle. Stopper and shake. Care should be used in handling bromine as the liquid or its fumes cause serious burns.

17. **Ammonium Chloride (10 Per Cent).**—Dissolve about 100 grams C.P. ammonium chloride in distilled water and make up to 1 liter.

18. **Saturated Ammonium Oxalate.**—Place about 50 grams of C.P. ammonium oxalate in a liter bottle and fill with distilled water. Shake and let stand until almost all of the crystals are dissolved. (Use the supernatant liquor.)
19. **Sulfuric Acid (2 Per Cent).**—Add 20 ml. of concentrated sulfuric acid to about 1 liter of distilled water.
20. **Standard Potassium Permanganate (0.125N).**—Dilute to 1 liter with distilled water the exact quantity of stock standard potassium permanganate solution (*E*), as determined by the following formula:

$$\frac{125}{\text{Normality of stock } KMnO_4} = \text{ml. stock } KMnO_4\text{, necessary to produce 1 liter of 0.125N } KMnO_4$$

21. **Disodium Phosphate (10 Per Cent).**—Dissolve about 100 grams of C.P. disodium phosphate (Na_2HPO_4) in distilled water and make up to 1 liter.
22. **Barium Chloride (10 Per Cent).**—Dissolve about 100 grams of C.P. barium chloride in distilled water and make up to 1 liter.
23. **Barium Hydroxide (Saturated).**—Place about 70 grams of barium hydroxide in a liter bottle. Almost fill with distilled water and shake for some time. Allow to settle and use the clear supernatant liquor.
24. **Platinic Chloride (10 Per Cent).**—Dissolve 10 grams of platinic chloride ($PtCl_4$) in distilled water and make up to 100 ml.
25. **Alcohol (80 Per Cent).**—Dilute 840 ml. of 95 per cent pure grain alcohol to 1 liter with distilled water.
26. **Permanent Iron Standards.** *Solution No.* 1.—Dissolve 0.400 gram of potassium chloroplatinate (K_2PtCl_6) in a small amount of distilled water, add 20 ml. concentrated hydrochloric acid and dilute to 100 ml. with distilled water.

    Solution No. 2.—Dissolve 4.800 grams of cobaltous chloride ($CoCl_2 \cdot 6H_2O$) in a small amount of distilled water, add 20 ml. of concentrated hydrochloric acid and make up to 100 ml. with distilled water. Plate the quantities of each solution as given in the following table

in 100-ml. Nessler tubes and dilute to the mark with distilled water.

Ml. solution No. 1	Ml. solution No. 2	Milligrams of iron
1.00	0.60	0.01
2.25	1.20	0.02
3.30	1.85	0.03
4.65	2.75	0.04
5.75	3.65	0.05
8.85	6.60	0.075
11.30	10.00	0.10
14.70	12.80	0.125
16.85	15.10	0.15

27. **Standard Iron Solution.**—Dissolve 0.7022 gram of ferrous ammonium sulfate crystals [$FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$] in about 50 ml. of distilled water and 20 ml. of concentrated sulfuric acid. Warm and add potassium permanganate solution (28) until a slight pink color persists. Dilute to 1 liter with distilled water. 1 ml. = 0.1 mg. Fe
28. **Potassium Permanganate Reagent.**—Dissolve about 6.32 grams of potassium permanganate in distilled water and make up to 1 liter. This is about 0.2N.
29. **Potassium Thiocyanate.**—Dissolve 20 grams of potassium thiocyanate in distilled water and make up to 1 liter.
30. **Dilute Methyl Orange.**—Dilute 5.9 ml. of methyl orange indicator (4) to 100 ml. with distilled water.
31. **Potassium Ferricyanide.**—Dissolve about 0.5 gram of crystalline potassium ferricyanide in distilled water and make up to 100 ml. This solution does not keep well and must be freshly prepared.
32. **Standard Ferrous Ammonium Sulfate.**—Dissolve 0.7022 gram of crystalline ferrous ammonium sulfate [$FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$] in freshly boiled distilled water to which 10 ml. of dilute sulfuric acid (30) has been added. Dilute to 1 liter with boiled distilled water. (This solution will not keep.) 1 ml. = 0.1 mg. Fe
33. **Standard Manganous Sulfate Solution.**—Dissolve 0.2873 gram of potassium permanganate in 100 ml. of distilled

water. Acidify with sulfuric acid and heat to boiling. Slowly add a dilute solution of oxalic acid until the color is just discharged. Cool and dilute to 1 liter. 1 ml. = 0.1 mg. Mn

34. **Nitric Acid Reagent.**—Add 100 ml. of concentrated nitric acid to 100 ml. of distilled water. If brown, bubble air through the solution in the hood until colorless.

35. **Silver Nitrate Reagent.**—Dissolve about 20 grams of silver nitrate in distilled water and make up to 1 liter.

36. **Standard Alum Solution.**—Dissolve about 2.5 grams of aluminum sulfate crystals [$Al_2(SO_4)_3 \cdot 18H_2O$] in boiled distilled water, add about 2 ml. of dilute sulfuric acid (63) and make up to 1 liter. This is a stock solution and the standard solution is prepared from it as follows:

a. Pipette two 50-ml. portions of the well-mixed solution into 250-ml. beakers.

b. Make distinctly alkaline to litmus with ammonium hydroxide.

c. Cover and boil gently until the odor of ammonia is slight.

d. Filter through quantitative filter paper and wash with hot distilled water until the filtrates give no white cloudiness when treated with a drop of barium chloride (22).

e. Ignite, cool and weigh two crucibles.

f. Place the filter papers in the crucibles, ignite, cool and weigh. The gain in weight is the weight of Al_2O_3 in a 50-ml. portion. The two weights should check.

The standard alum solution is made as follows: Measure a portion of the standardized stock solution, as determined by the formula below, into a liter volumetric flask. Add 2 ml. of dilute sulfuric acid (63) and dilute to the mark with boiled distilled water.

$$\frac{0.5}{\text{Gm. } Al_2O_3 \text{ in 50 ml. stock alum}} = \text{ml. standardized stock alum solution required}$$

$$1 \text{ ml} = 0.01 \text{ mg. } Al_2O_3$$

37. **Permanent Alum Standards.**—The standards prepared according to the method given in the residual alum determination may be sealed in the phosphoric acid

flasks. These standards will be more permanent if a good surface water is used instead of distilled water for diluting the standard alum solution in the flasks. This water should first be stored for about 4 days and filtered.

38. **Alizarin Red S. (0.3 Per Cent).**—Pour 5.5 ml. of concentrated sulfuric acid into about 95 ml. of distilled water. Dissolve about 0.3 gram of alizarin red S. (alizarin sodium monosulfonate) in the sulfuric acid solution.

39. **Sodium Bicarbonate (Saturated).**—Mix about 100 grams of sodium bicarbonate with 1 liter of distilled water. Stir until the solution is saturated. Filter and add about 20 ml. of distilled water to the filtrate.

40. **Acetic Acid (50 Per Cent).**—Dilute 50 ml. of glacial acetic acid with 50 ml. of boiled distilled water.

41. **Color Standards.**—Dissolve 0.249 gram of potassium chloroplatinate (K_2PtCl_6) and 0.200 gram of crystallized cobalt chloride ($CoCl_2 \cdot 6H_2O$) in a small amount of distilled water. Add 20 ml. of concentrated hydrochloric acid and dilute to 200 ml. with distilled water. Place 1, 2, 3, etc., to 10 ml. of this solution in 100-ml. Nessler tubes and dilute to the mark. These represent colors of 5, 10, 15, etc.

42. **Turbidity Standards, 5 to 25.**—Pour slowly and with constant stirring 10 grams of Fuller's earth, or 2 grams of bentonite, into a beaker containing 200 to 300 ml. of distilled water. Transfer this suspension to a liter bottle and nearly fill with distilled water. Shake vigorously several times and allow to stand overnight. Withdraw the supernatant liquor into another bottle and test the turbidity with the Jackson turbidimeter. From this stock suspension make up, in liter clear-glass bottles, suspensions having turbidities of 5, 10, 15, 20 and 25 by diluting an appropriate amount with distilled water. To determine the amount of stock suspension necessary to use in making up the standards, apply the following formula:

$$\frac{\text{Turbidity desired} \times 1{,}000}{\text{Turbidity of stock suspension}} = \text{ml. stock suspension necessary to make 1 liter of standard}$$

43. **Turbidity Standards, Baylis Turbidimeter.**—Make these standards in the same manner as described above. Standards having turbidities of 0.2, 0.5, 0.75, 1.0, 1.5 and 2.0 should be made.

44. **Ammonia-free Water.**—Add 2 ml. of concentrated sulfuric acid to each liter to distilled water and redistill.

45. **Permanent Ammonia Standards.** *Solution No.* 1.—Dissolve exactly 0.400 gram of potassium chloroplatinate (K_2PtCl_6) in a small amount of distilled water. Add 20 ml. of concentrated hydrochloric acid and dilute to 200 ml.

Ml. solution No. 1	Ml. solution No. 2	Mg. ammonia nitrogen
2.8	0.0	0.002
4.7	0.1	0.004
5.9	0.2	0.007
7.7	0.5	0.010
9.9	1.1	0.014
12.7	2.2	0.020
15.0	3.3	0.025
17.3	4.5	0.030
19.7	7.1	0.040
20.0	10.4	0.050

Solution No. 2.—Dissolve exactly 1.200 grams of cobaltous chloride ($CoCl_2 \cdot 6H_2O$) in a small amount of distilled water, add 10 ml. of concentrated hydrochloric acid and make up to 100 ml.

Measure into 100-ml. Nessler tubes the volumes of each solution as given above and dilute to the mark with distilled water. Stopper tightly and keep in dark place.

46. **Standard Ammonium Chloride.**—Dissolve 3.820 grams of pure ammonium chloride in 1 liter of distilled water. Mix thoroughly and dilute 10 ml. of this solution to 1 liter with ammonia-free water (44).

1 ml. = 0.010 mg. of N = 0.01216 mg. of NH_3

47. **Nessler Reagent.**—Dissolve about 50 grams of potassium iodide in 35 ml. of cold ammonia-free water (44). Add

a saturated solution of mercuric chloride until a slight precipitate persists. Add 400 ml. of a 50 per cent solution of potassium hydroxide. Dilute to 1 liter, allow to settle and decant.

48. **Alkaline Potassium Permanganate.**—Boil about 600 ml. of distilled water in a large evaporating dish for 10 min. Add about 8 grams of potassium permanganate and stir until completely dissolved. Dissolve about 180 grams of sodium hydroxide in 500 ml. of ammonia-free water (44) and let stand until any insoluble matter settles. Add 400 ml. of the clear supernatant sodium hydroxide solution to the potassium permanganate solution in the dish and make up to about 1,200 ml. with ammonia-free water. Boil until the volume has been reduced to about 1 liter.

49. **Phosphate Buffer Solution (pH 7.4).**—Dissolve 14.300 grams of monopotassium phosphate (KH_2PO_4) and 90.150 grams of dipotassium phosphate ($K_2HPO_4 \cdot 3H_2O$) in distilled water and make up to 1 liter.

50. **Standard Sodium Nitrite.**—Dissolve 1.100 grams of silver nitrite in nitrite-free water. Add a solution of sodium chloride until the silver chloride is completely precipitated. Dilute to 1 liter and allow to settle. Dilute 100 ml. of the clear supernatant liquor to 1 liter and mix well. Finally dilute 50 ml. of the latter solution to 1 liter. Mix and add a few drops of chloroform.

1 ml. = 0.0005 mg. of N = 0.00164 mg. of NO_2

51. **Sulfanilic Acid.**—Dissolve about 8.0 grams of pure sulfanilic acid in 750 ml. of water and 250 ml. of glacial acetic acid.

52. **Alpha-naphthylamine.**—Dissolve about 5.0 grams of alpha-naphthylamine in a mixture of 750 ml. of water and 250 ml. of glacial acetic acid. Filter through glass wool or cotton.

53. **Standard Silver Sulfate.**—Dissolve 4.397 grams of C.P. silver sulfate in 1 liter of distilled water.

1 ml. = 1 mg. Cl

54. **Phenoldisulfonic Acid.**—Dissolve about 25 grams of pure phenol in 150 ml. of concentrated sulfuric acid and add 75 ml. of fuming sulfuric acid. Heat for 2 hr. over a boiling-water bath.

55. **Sodium Hydroxide (Approximately 12N).**—Dissolve about 480 grams of pure sodium hydroxide in distilled water and make up to about 1 liter.

56. **Standard Nitrate Solution.**—Dissolve 0.7216 gram of pure potassium nitrate in 1 liter of distilled water. Evaporate 50 ml. of this solution to dryness on the water bath. Moisten the residue with 2 ml. of phenoldisulfonic acid (54) rubbing it well into the residue to insure intimate contact. Dilute to 500 ml. with distilled water.

1 ml. = 0.01 mg. of N = 0.04426 mg. of NO_3

57. **Manganous Sulfate.**—Dissolve about 480 grams of manganous sulfate crystals ($MnSO_4 \cdot 4H_2O$) or 400 grams of $MnSO_4 \cdot 2H_2O$ in sufficient distilled water to make 1 liter.

58. **Alkaline Potassium Iodide.**—Dissolve about 500 grams of sodium hydroxide, 20 grams of sodium azide and 150 grams of potassium iodide in sufficient distilled water to make 1 liter. (The sodium azide may be omitted if it is certain that nitrite interference will not be encountered in the dissolved-oxygen test.)

59. **Standard Sodium Thiosulfate (0.025N).**—Dilute the calculated amount of stock standard sodium thiosulfate solution (*D*) to 1 liter with distilled water. Add 5 ml. of chloroform. Make up fresh every 2 weeks.

$$\frac{25}{\text{Normality of stock standard } Na_2S_2O_3} = \text{ml. stock standard } Na_2S_2O_3 \text{ to make 1 liter of 0.025N } Na_2S_2O_3$$

Note.—The 0.025N solution can be made up directly by dissolving 6.205 grams of sodium thiosulfate in a liter of distilled water. If this procedure is followed, it is important that crystals which have become white, owing to loss of water of crystallization, should not be used.

60. **Starch Indicator.**—Make a thin paste of about 2 grams of starch in cold water. Pour into 200 ml. boiling water and stir. When cool add a few drops of chloroform.

61. **Hydrochloric Acid (1 to 3).**—Add 250 ml. of concentrated hydrochloric acid to 750 ml. of distilled water. This is about 3N.
62. **Potassium Oxalate.**—Dissolve about 20.0 grams of potassium oxalate in sufficient water to make 1 liter.
63. **Sulfuric Acid (1 to 3).**—Add slowly with constant stirring 250 ml. of concentrated sulfuric acid to 750 ml. of distilled water. This is about 9.6N.
64. **Standard Ammonium Oxalate (0.0125N).**—Dissolve exactly 0.8881 gram of pure ammonium oxalate crystals in 1 liter of distilled water.
65. **Standard Potassium Permanganate (0.0125N).**—Dissolve 0.400 gram of potassium permanganate in 1 liter of distilled water and titrate against the ammonium oxalate solution (64) as follows:

a. Place 100 ml. of distilled water into an Erlenmeyer flask and add 10 ml. of 1 to 3 sulfuric acid (63) and 10 ml. of the potassium permanganate solution.
b. Place in a boiling-water bath for 30 min.
c. Add 10 ml. of ammonium oxalate solution (64) and then add the potassium permanganate solution, drop by drop, from a burette, until the first slight permanent pink color. (No record of the volume of permanganate solution used up to this point need be made.)
d. Heat almost to boiling, add exactly 10 ml. of ammonium oxalate solution (64).
e. Titrate with the permanganate solution to the first pink color. Record the ml. of permanganate solution used.
f. Adjust the permanganate solution so that 1 ml. of oxalate = 1 ml. of permanganate.

66. **Orthotolidine Solution.**—Place about 1.0 gram of orthotolidine in a large mortar and add 5 ml. of 1 to 4 hydrochloric acid (100 ml. of concentrated hydrochloric acid in 400 ml. of distilled water). Grind to a thin paste and add 150 ml. of distilled water. Stir until the orthotolidine is dissolved. Transfer to a 1-liter graduated cylinder and make up to 505 ml. with distilled water. Add the remaining 495 ml. of 1 to 4 hydrochloric acid.

66A. **Orthotolidine Solution for Break Point.**—Place about 1 gram of orthotolidine in a large mortar and add 5 ml. of 1 to 9 hydrochloric acid (10 ml. of concentrated hydrochloric acid in 90 ml. of distilled water). Grind to a thin paste, add 150 ml. of distilled water, stir until the orthotolodine is dissolved and make up to 1 liter with distilled water.

67. **Color Standards for Chlorine.** *Solution No.* 1.—Dissolve 1.500 grams of copper sulfate and 1 ml. of concentrated sulfuric acid in distilled water and make up to 100 ml.

Solution No. 2.—Dissolve 0.250 gram of potassium dichromate and 1 ml. of concentrated sulfuric acid in distilled water and make up to 1 liter. Place the quantities of the two solutions indicated in the following table in 100-ml. Nessler tubes and make up to the mark with distilled water. Stopper and keep in a dark place.

Ml. solution No. 1	Ml. solution No. 2	P.p.m. Cl
0.4	5.5	0.05
1.8	10.0	0.10
1.9	20.0	0.20
1.9	30.0	0.30
2.0	38.0	0.40
2.0	45.0	0.50
2.0	58.0	0.70
2.0	72.0	1.00

68. **Standard Sodium Thiosulfate (0.001N).**—Dilute the calculated amount of stock standard sodium thiosulfate solution (*D*) to 1 liter with distilled water. Make up fresh every few days.

$$\frac{1}{\text{Normality of stock standard } Na_2S_2O_3} = \text{ml. stock standard } Na_2S_2O_3 \text{ required to make 1 liter of 0.001N } Na_2S_2O_3$$

69. **Standard Iodine Solution (0.025N).**—Dissolve about 2.0 grams of potassium iodide crystals in a small quantity of hot, boiled distilled water. Cool, add 3.173 grams

of iodine and make up to 1 liter with distilled water. Titrate 25 ml. of this solution with the 0.025N sodium thiosulfate (59) using starch (60) as an indicator. Adjust the solution so that 1 ml. is equivalent to 1 ml. of the thiosulfate by use of the following equation:

$$\frac{25{,}000}{\text{Ml. thiosulfate used}} = \text{ml. iodine solution necessary to produce 1 liter of 0.025N iodine solution}$$

70. **Cleaning Solution.**—Dissolve about 100 grams of commercial potassium dichromate in 375 ml. water and make up to 1 liter with concentrated sulfuric acid. Add the acid to the water solution with constant stirring. The strength of this solution may be restored by further additions of concentrated sulfuric acid.
71. **Diluting Water for Biochemical Oxygen Demand (B.O.D.).** Add about 6 grams of sodium bicarbonate to 5 gal. of distilled water. Aerate by bubbling air through the water until the dissolved-oxygen content is above 7.0 p.p.m. This may require several days. Store for at least 2 weeks before using.
72. **Calibration of Bottles.**—Weigh the bottle empty and again when completely full of distilled water. The difference in weight in grams is the capacity of the bottle in ml.
73. **Methylene Blue.**—Dissolve about 1.0 gram of methylene blue (Merck's double zinc salt) in 1 liter of distilled water.
74. **Copper Sulfate Solution (10 Per Cent).**—Dissolve about 100 grams of copper sulfate crystals in distilled water and make up to 1 liter.
75. **Chlorine Water.**—This solution should contain 1.0 gram of chlorine per liter. Make a slightly stronger solution by passing chlorine gas into 1 liter of distilled water. (Chlorine compounds may be used, instead of chlorine gas, for making the chlorine water.) Standardize this solution as follows:

a. Dissolve about 2 grams of potassium iodide in 50 ml. of distilled water in an Erlenmeyer flask and add 2 ml. of glacial acetic acid.

b. Pipette 5 ml. of the chlorine solution into the flask.

c. Titrate with 0.025N sodium thiosulfate solution (59) using starch (60) as an indicator near the end of the titration. Calculate the grams of chlorine in 1 liter of solution by multiplying the ml. of thiosulfate by 0.1773.

d. Dilute the chlorine solution so as to contain 1 gram of chlorine per liter, as follows:

$$\frac{1{,}000}{\text{Gm. of chlorine per liter}} = \text{ml. of chlorine solution to give 1 liter of solution containing 1 gm. Cl}$$

76. Hydrochloric Acid (Approximately 1N).—Dilute 93 ml. of concentrated hydrochloric acid with sufficient distilled water to make 1 liter of solution.

77. Molybdate Solution.—Dissolve about 100 grams of molybdic acid in dilute ammonium hydroxide (144 ml. of concentrated ammonium hydroxide and 271 ml. of water). Pour this solution slowly with constant stirring into dilute nitric acid (489 ml. of concentrated nitric acid and 1,148 ml. of distilled water). Keep the mixture in a warm place for several days or until a portion heated to 40°C. deposits no yellow precipitate of ammonia phosphomolybdate. Decant the solution from any sediment and preserve in a glass-stoppered bottle. Before using, add 5 ml. of concentrated nitric acid to 100 ml. of the molybdate solution and filter.

78. Standard Sodium Hydroxide (0.3238N).—Dilute the calculated amount of stock standard sodium hydroxide (*B*) to 1 liter with boiled distilled water.

$$\frac{323.8}{\text{Normality of stock standard NaOH}} = \text{ml. of stock standard NaOH necessary to make 1 liter of 0.3238N NaOH}$$

79. Standard Hydrochloric Acid (0.3238N).—Dilute the calculated amount of stock standard hydrochloric acid (*C*) to 1 liter with distilled water.

$$\frac{323.8}{\text{Normality of stock standard HCl}} = \text{ml. of stock standard HCl necessary to make 1 liter of 0.3238N HCl}$$

80. **Salicylic Acid Mixture.**—Dissolve about 1 gram of salicylic acid in 30 ml. of concentrated sulfuric acid.
81. **Boric Acid.**—Dissolve 40 grams of boric acid in 1 liter of distilled water.
82. **Standard Hydrochloric Acid (0.5N).**—Dilute the calculated amount of stock standard hydrochloric acid (*C*) to 1 liter with distilled water.

$$\frac{500}{\text{Normality of stock standard HCl}} = \text{ml. of stock standard HCl necessary to make 1 liter of 0.5N HCl}$$

83. **Potassium Hydroxide.**—Dissolve about 100 grams of pure potassium hydroxide in 200 ml. of distilled water.
84. **Phenolphthalin for Cyanides (Not Phenolphthalein).**—Dissolve about 0.5 gram of phenolphthalin (Eastman Kodak Co.) in about 150 ml. of dilute sodium hydroxide (about 1 gm. NaOH in 150 ml. of distilled water). Keep in a dark place.
85. **Copper Sulfate (0.05 Per Cent).**—Dissolve about 0.5 gram of copper sulfate crystals in 1 liter of distilled water.
86. **Sodium Hydroxide (0.2N).**—Dilute the calculated amount of stock standard sodium hydroxide (*B*) to 1 liter with boiled distilled water.

$$\frac{200}{\text{Normality of stock standard NaOH}} = \text{ml. stock standard NaOH necessary to make 1 liter of 0.2N NaOH}$$

87. **Thymolphthalein.**—Prepare a stock solution by grinding about 0.4 gram of thymolphthalein with 17.2 ml. of 0.05N sodium hydroxide (12) and make up to 100 ml. with distilled water. For use, dilute 10 ml. of this stock solution to 100 ml. with distilled water.
88. **Buffer Solution for Phenols.**—Dissolve about 6.200 grams of boric acid and 7.450 grams of potassium chloride in 1,500 ml. of distilled water. Adjust with 0.2N sodium hydroxide (86) to such a strength that 5 ml. when added to 100 ml. of the distilled water will give a pH of 9.6. This buffer is to be used with samples of low phenol content.

89. Standard Phenol Solution.—First prepare a stock standard phenol solution by dissolving 1 gram of pure phenol in 1 liter of distilled water. Standardize as follows:

a. Pipette 50 ml. of the well-mixed phenol solution into an Erlenmeyer flask.

b. Add 50 ml. of 0.1N bromine (93) and 5 ml. of concentrated hydrochloric acid.

c. Allow to stand in cool water for 15 min. and add about 2 grams of potassium iodide crystals.

d. Titrate with 0.1N sodium thiosulfate (92) using starch (60) as an indicator near the end of the titration. Record the ml. of thiosulfate used.

(50 − ml. 0.1N thiosulfate) × 0.0312 = mg. phenol per ml.

To make a standard solution in which 1 ml. = 0.01 mg. of phenol, dilute the stock standard solution with distilled water as follows:

$$\frac{10}{\text{Mg. phenol per liter}} = \text{ml. of stock standard phenol solution necessary to make 1 liter of standard phenol solution}$$

90. 2,6-Dibromoquinonechloroimide.—Dissolve about 0.1 gram of 2,6-dibromoquinonechloroimide in 25 ml. of 95 per cent ethyl alcohol and place in a tightly stoppered brown bottle. This stock solution must be made up fresh every 3 days. Before the test is made, dilute 10 ml. of the stock solution to 200 ml. with distilled water. This solution will not last over 30 min.

91. Sodium Hydroxide (20 Per Cent).—Dissolve about 100 grams of sodium hydroxide in 400 ml. of distilled water.

92. Sodium Thiosulfate (0.1N).—Dilute to 1 liter with distilled water, the calculated amount of stock standard sodium thiosulfate solution (*D*).

$$\frac{100}{\text{Normality of stock standard } Na_2S_2O_3} = \text{ml. stock standard } Na_2S_2O_3 \text{ necessary to make 1 liter of 0.1N } Na_2S_2O_3$$

93. Bromine (0.1N).—Dissolve exactly 2.784 grams of potassium bromate and about 10.0 grams of potassium

bromide in distilled water and make up to 1 liter. Standardize as follows:

a. Pipette 25 ml. of the well-mixed bromine solution into an Erlenmeyer flask.

b. Add about 75 ml. of distilled water and 5 ml. of concentrated hydrochloric acid.

c. Add 2 grams of potassium iodide crystals and titrate against the 0.1N sodium thiosulfate solution (92) using starch (60) near the end of the titration.

d. Adjust the solution by diluting with distilled water in the proportion indicated below so that 1 ml. of bromine solution = 1 ml. of thiosulfate solution.

$$\frac{25{,}000}{\text{Ml. of thiosulfate used}} = \text{ml. of bromine solution necessary to make 1 liter of 0.1N bromine}$$

94. Potassium Permanganate (0.1N).—Dilute the calculated amount of stock standard potassium permanganate solution (*E*) to 1 liter with distilled water.

$$\frac{100}{\text{Normality of stock standard } KMnO_4} = \text{ml. of stock standard } KMnO_4 \text{ necessary to make 1 liter of 0.1N } KMnO_4$$

95. Sulfuric acid (0.1N).—Dilute the calculated amount of the stock standard sulfuric acid solution (*A*) to 1 liter with distilled water.

$$\frac{100}{\text{Normality of stock standard } H_2SO_4} = \text{ml. of stock standard } H_2SO_4 \text{ necessary to make 1 liter of 0.1N } H_2SO_4$$

96. Approximately 0.2N Hydrochloric Acid.—Dilute 17 ml. of concentrated hydrochloric acid to 1 liter with distilled water.

97. Approximately 0.2N Sodium Hydroxide.—Dissolve about 8 grams of sodium hydroxide in 1 liter of distilled water.

98. Phosphoric Acid (10 Per Cent).—Dilute 118 ml. of 85 per cent phosphoric acid to 1 liter with distilled water.

99. Phosphoric Acid (50 Per Cent).—Dilute 118 ml. of 85 per cent phosphoric acid to 200 ml. with distilled water.

100. **Copper Sulfate Solution.**—Dissolve about 0.05 gram of copper sulfate in 1 liter of distilled water.
101. **Strong Buffer Solution for Phenol.**—Dissolve about 6.200 grams of boric acid and 7.450 grams of potassium chloride in 1,500 ml. of distilled water. Adjust with 0.2N sodium hydroxide (86) to such a strength that 1 ml. when added to 100 ml. of distilled water will give a pH of 9.6. This buffer solution is to be used with samples having high phenol contents.
102. **Ferric Chloride (10 Per Cent).**—If anhydrous ferric chloride is used, dissolve about 100 grams of the salt in 1 liter of distilled water. If crystalline ferric chloride is used, dissolve 167 grams of the crystals in 1 liter of water.
103. **Sulfanilic Acid.**—Add 1.91 grams of sulfanilic acid to 250 ml. of distilled water.
104. **Sodium Nitrite.**—Add 1.20 grams of sodium nitrite to 250 ml. of distilled water. This material is hygroscopic. Desiccated material must be used.
105. **Calcium Reagent.**—Dissolve 70 grams of ammonium chloride and 20 grams of oxalic acid in distilled water and make up to 1 liter.
106. **Sodium Hydroxide (8 Per Cent).**—Dissolve 8 grams of sodium hydroxide in 92 ml. of distilled water.
107. **Carbon-treated, Phenol-free Water.**—Add 0.03 gram of carbon to 3,000 ml. of distilled water. Stir for 1 hr. on the mechanical stirrer at about 250 r.p.m. Filter through No. 40 Whatman filter paper using a Büchner funnel with the aid of suction. Discard the first 100 ml.
108. **Stock Phenol Solution.**—Dissolve 1 gram of pure phenol in 1,000 ml. of distilled water. Standardize the solution according to the procedure given on page 189 and adjust so that 1 ml. contains 1 mg. of phenol.
109. **Standard Phenol Solution.**—Add 1 ml. of the stock phenol solution (108) to 1,000 ml. of carbon-treated phenol-free water (107). A new standard solution should be made each time the test is made. One milliliter of this solution when diluted to 100 ml. gives a solution containing 10 p.p.b. (parts per billion) phenol.

110. Ammonia Water (3 Per Cent).—Add 3 ml. of concentrated ammonium hydroxide to 97 ml. of distilled water.

111. Standard Potassium Iodide Solution.—Dissolve exactly 0.026 gram of potassium iodide in 1 liter of distilled water. 1 ml. = 0.02 mg. iodide.

112. Standard Sodium Fluoride Solution.—Dissolve 0.2210 gram sodium fluoride in distilled water and make up to exactly 100 ml. Dilute exactly 10 ml. of this solution to exactly 1 liter with distilled water. 1 ml. = 0.01 mg. fluoride.

113. Zirconium—Alizarin Reagent.—Dissolve 1.0 gram of zirconium oxychloride ($ZrOCl_2 \cdot 8H_2O$) in distilled water and make up to 100 ml. Dissolve 0.2 gram of alizarin sodium monosulfonate in distilled water and make up to 100 ml. To 25 ml. of the zirconium oxychloride solution add slowly and with constant stirring 25 ml. of the alizarin sodium monosulfonate solution. Allow to stand overnight and filter, if necessary. Add 50 ml. of distilled water.

114. Chemicals for Jar Test.—Dissolve 17.14 grams of alum, lime, soda ash, ferric chloride or any other chemical used, each in 1 liter of distilled water. Then 1 ml. of this chemical solution in 1 liter of the sample = a dose of 1 g.p.g.

115. Ammonium Carbonate (10 Per Cent).—Dissolve about 100 grams of ammonium carbonate in water and make up to 1 liter.

116. Standard 0.03424N Sulfuric Acid.—Dilute the exact quantity of stock standard sulfuric acid solution (*A*) as determined by the following formula to 1 liter with distilled water.

$$\frac{34.24}{\text{Normality of stock } H_2SO_4} = \text{ml. stock } H_2SO_4 \text{ necessary to prepare 1 liter of 0.03424N } H_2SO_4$$

117. Standard 0.02N Sodium Carbonate.—Dissolve exactly 1.060 grams of anhydrous sodium carbonate in freshly boiled and cooled distilled water and make up to 1 liter.

118. Standard 0.03424N Sodium Carbonate.—Dissolve exactly 1.923 grams of anhydrous sodium carbonate in freshly boiled and cooled distilled water and make up to 1 liter.

119. Benzidine Hydrochloride (2 Per Cent).—Place 20 grams of benzidine hydrochloride in an agate mortar and work into a smooth paste with a small amount of distilled water. Wash the paste into a liter flask, add 10 ml. of concentrated hydrochloric acid and make up to the mark with distilled water.

120. Standard 0.143N (N/7) Sodium Hydroxide.—Dilute the exact quantity of stock standard sodium hydroxide (*B*) as determined by the following formula to 1 liter with freshly boiled and cooled distilled water.

$$\frac{143}{\text{Normality of stock NaOH}} = \text{ml. of stock NaOH necessary to prepare 1 liter of 0.143N NaOH}$$

121. Xyelene Cyanole-Methyl Orange.—Dissolve 0.2 gram of xylene cyanole and 1 gram of methyl orange in distilled water and make up to 1 liter.

122. Standard 0.121N Sodium Hydroxide.—Dilute the exact quantity of stock standard sodium hydroxide (*B*) as determined by the following formula to 1 liter with freshly boiled and cooled distilled water.

$$\frac{121}{\text{Normality of stock NaOH}} = \text{ml. of stock NaOH necessary to prepare 1 liter of 0.121N NaOH}$$

123. Standard 0.0172N (N/58.3) Silver Nitrate.—Dissolve exactly 2.9220 grams of silver nitrate in distilled water and make up to 1 liter.

124. Sodium Carbonate (10 Per Cent).—Dissolve 10 grams of sodium carbonate (soda ash) in 90 ml. of distilled water.

125. Borate Mixture.—Dissolve 7.440 grams of boric acid in exactly 500 ml. of 0.2N sodium hydroxide (86) and make up to 1 liter with distilled water.

126. Calcium Chloride Solution.—Dissolve 7.55 grams of calcium chloride (anhydrous basis) in distilled water and make up to 100 ml.

127. Dilute Hydrochloric Acid.—Mix 60 ml. of concentrated hydrochloric acid and 40 ml. of distilled water.

128. Permanent Standards for Silica Test.—Make up a stock solution by dissolving exactly 0.530 gram of potassium chromate (K_2CrO_4) in distilled water and making up to 1 liter. The color standards are prepared by diluting the quantities of stock solution given in the following table to 103 ml. in 100-ml. Nessler tubes.

Ml. K_2CrO_4 stock solution	P.p.m. SiO_2 represented by standard	P.p.m. SiO_2 in sample treated according to procedure
1.0	1.89	2.78
2.0	3.77	5.55
3.0	5.66	8.31
4.0	7.54	11.10
5.0	9.43	13.85
6.0	11.32	16.60
7.0	13.21	19.45
8.0	15.08	22.17
9.0	16.97	24.90
10.0	18.85	27.80
11.0	20.74	30.50
12.0	22.64	33.30
13.0	24.53	36.00
14.0	26.42	38.80
15.0	28.31	41.50

SECTION III

GENERAL DISCUSSION OF CHEMISTRY AND RELATED TOPICS

DIVISION I

CHEMISTRY AND DISCUSSION OF METHODS

FOREWORD

The preceding sections of this book are confined to instructions for making certain chemical analytical determinations on water, sewage and trade wastes. These instructions are sufficiently detailed and clear so that they can be followed without referring to other explanations. It is necessary in many instances for the laboratory worker to understand a certain amount of chemistry in order that he may intelligently apply even detailed instructions. A certain facility in carrying through instructions for the procedure for analytical determinations may be acquired by practice, but the analyst should not be content until he understands the reasons upon which the procedures have been formulated. Many laboratory workers will no doubt find that they are thoroughly familiar with the material contained in this section as it is intended only as a very elementary treatise of the chemistry which is essential in order to acquire a fair understanding of the analytical determinations given in this book.

This section will cover explanations of units used in chemistry, the writing of formulas and equations, methods of expressing results, reactions involved in the determinations and other fundamental relations.

FUNDAMENTALS OF CHEMISTRY

Units of Measurement.

The ordinary units of measurement used in laboratory work are based on the metric system. It is sometimes necessary to convert values from the metric system to the English system or

vice versa. Convenient conversion values are given later in this discussion.

The unit of weight is the kilogram. There are 1,000 grams in 1 kilogram and 1,000 milligrams in 1 gram. Thus, there are 1,000,000 milligrams in 1 kilogram.

The unit of volume is the liter. It is the volume of 1 kilogram of pure water at a temperature of 4°C. and 76 cm. of mercury pressure. There are 1,000 milliliters in 1 liter. Therefore, 1 milliliter of water weighs 1 gram. This relation of weight and volume makes the use of metric units very convenient in the laboratory. This relation is not quite true for other temperatures and pressures but the effect of these factors may be neglected in ordinary work. The term "cubic centimeter" (cc.) is often used in place of "milliliter" (ml.).

The unit of length is the meter. There are 100 centimeters (cm.) in the meter and 10 millimeters (mm.) in the centimeter. There are thus 1,000 millimeters in 1 meter.

Temperatures are generally recorded in degrees centigrade. Zero degree of the centigrade thermometer is the freezing point of water and 100 degrees is the boiling point of water. These values correspond to 32 degrees and 212 degrees, respectively, on the Fahrenheit scale.

The results of the chemical analysis of water and sewage are usually expressed in parts per million (p.p.m.) by weight. One liter of water or sewage, with its contained impurities, is assumed to weigh 1 kilogram as it is used in the laboratory. It will be noticed from the weight relations given above that 1 p.p.m. is the same as 1 milligram per liter. This is a convenient relation to have in mind when performing certain calculations. In special cases of waters containing large amounts of impurities, the results obtained should be divided by the specific gravity of the sample.

The results may also be expressed in grains per gallon. One grain per gallon (g.p.g.) is equivalent to 17.12 p.p.m. The following conversion factors will be found convenient.

1 milligram per liter = 1 part per million (p.p.m.)
1 grain per gallon (g.p.g.) = 17.12 p.p.m.
1 part per million = 8.34 pounds per million gallons
1 liter = 0.2642 gallons
1 kilogram = 2.205 pounds

1 pound = 453.6 grams

1 degree centigrade = 1.8 degrees Fahrenheit

Other useful conversion factors will be found on page 213 in Table 6.

Chemistry.

All matter is made up of one or more elements. The elements are fundamental substances which cannot be divided into simpler substances. Compounds are substances consisting of elements chemically combined. The elements are designated by abbreviations or symbols such as O for oxygen, H for hydrogen, S for sulfur, etc.

The atoms of an element have a definite weight called the atomic weight. The atomic weights are based on oxygen as 16 in which case the atomic weight of hydrogen is 1.008. A list of the elements encountered in water and sewage analyses and their atomic weights is given in Table 3 on page 208.

Elements combine in definite proportions to form compounds. The make-up of a compound is indicated by its formula, which is a combination of the symbols of the elements. Thus, water is composed of two atoms of hydrogen and one atom of oxygen and its formula is H_2O. Sulfuric acid is composed of two atoms of hydrogen, one of sulfur and four of oxygen and its formula is H_2SO_4. The symbol for an element indicates one atom and likewise the formula for a compound indicates one molecule. Thus 2H indicates two atoms of hydrogen and $2H_2SO_4$ indicates two molecules of sulfuric acid. The molecular weight of a compound is equal to the sum of the atomic weights of the atoms in the molecule. Thus a molecule of water (H_2O) has a molecular weight of $(2 \times 1.008) + 16.00 = 18.016$.

The combining power or ratio in which atoms of an element combine with atoms of other elements is called valence. The valence of hydrogen is one. The valence of the other elements is determined by an examination of the formula of a compound in which the element occurs. Oxygen, for instance, has a valence of two since it occurs in water combined with two hydrogen atoms (H_2O). Chlorine has a valence of one since it occurs in HCl combined with one hydrogen atom.

Not all elements will combine to form compounds. For instance, hydrogen will combine with oxygen, chlorine and

nitrogen but will not combine with metals such as iron, calcium and sodium. The bond or force which holds elements together in compounds is electrical in nature and elements having like electrical tendencies will not combine. Thus, hydrogen and the metals are said to have positive electrical tendencies and chlorine, oxygen, etc., negative. The comparative value of these electrical forces constitutes the valence of the atoms. Hydrogen is said to have a positive valence of one, oxygen a negative valence of two, etc. The sum of the positive valences in any compound must be equal to the sum of the negative valences. Thus, in H_2SO_4, the oxygen has a total negative valence of eight and hydrogen positive two, leaving six positive for the sulfur. A few elements assume different valences under different conditions. Carbon may have positive valence of two or four in carbon monoxide (CO) and carbon dioxide (CO_2), respectively. Some elements encountered in water and sewage analysis may have either positive or negative valence. For instance, N has a negative valence of three in NH_3 and a positive valence of four in NO_2. Valences of elements are given in Table 3 on page 208.

The atomic and molecular weights are the basis of all quantitative chemical reactions and are used in all quantitative calculations. A knowledge of them is necessary in order to understand the calculations involved in the chemical determinations. For example, the formula for sulfuric acid is H_2SO_4. The molecular weight of H_2SO_4 and the percentage composition of the elements in this compound may be obtained by referring to the atomic weights as follows:

$$\begin{aligned}
\text{Hydrogen, 2 atomic weights} &= 2 \times 1.008 = 2.016 \\
\text{Sulfur, 1 atomic weight} &= 1 \times 32.06 = 32.060 \\
\text{Oxygen, 4 atomic weights} &= 4 \times 16.00 = \underline{64.000} \\
&\text{Molecular weight} = 98.076
\end{aligned}$$

The percentage by weight of hydrogen is $\frac{2.016}{98.076} \times 100 = 2.04$, that of sulfur is $\frac{32.06}{98.076} \times 100 = 32.69$ and that of oxygen is $\frac{64.00}{98.076} \times 100 = 65.36$.

The chemical reaction and the relative weights of the reacting substances are shown by writing an equation using the formula and molecular weights as follows:

$$\begin{array}{ccccccc} H_2SO_4 & + & 2NaOH & = & Na_2SO_4 & + & 2H_2O \\ 98.08 & & 2 \times 40.0 & & 142.06 & & 2 \times 18.02 \end{array}$$

This equation indicates that one molecule of sulfuric acid reacts with two molecules of sodium hydroxide to form one molecule of sodium sulfate and two molecules of water. The number of molecules and molecular weights written below the formulas indicate the weight ratios between the reacting substances. Thus 98.08 grams (pounds, tons or other weight units) of sulfuric acid reacts with 80.0 grams of sodium hydroxide to form 142.06 grams of sodium sulfate and 36.04 grams of water. These weight ratios may be used to solve problems similar to the one which follows.

Calculations.

How much sodium hydroxide will react with 100 lb. of sulfuric acid?
Let A = the lb. of sodium hydroxide.
Then

$$\frac{A}{100} = \frac{80.00}{98.08}$$

$$A = 100 \times \frac{80.00}{98.08} = 81.6 \text{ lb. of sodium hydroxide}$$

In all cases, knowing the equation for a given reaction, the weight ratios can be determined from the atomic weights of the elements and the formulas of the compounds involved. Since the weight ratios can be found, if the actual weight of any one compound entering into the reaction is known, the weights of all of the other compounds involved can be determined. A list of chemical formulas and equations encountered in water and sewage analysis is given in Tables 4 and 5, pages 209 and 211.

Problems.

1. Calculate the weight of $Al_2(SO_4)_3$ in 100 lb. of alum [$Al_2(SO_4)_3 \cdot 18H_2O$]. *Ans.* 51.3 lb.

2. How much ferric hydroxide is obtained from the action of 50 lb. of ferric chloride crystals ($FeCl_3 \cdot 6H_2O$) with calcium hydroxide? What weight of calcium hydroxide will be required? *Ans.* 19.7 and 20.6 lb.

3. What weight of carbon dioxide is obtained from burning 200 lb. of limestone (assuming limestone to be pure calcium carbonate)?

Ans. 88.0 lb.

4. What weight of calcium carbonate is produced by the action of sodium carbonate and 40 lb. of calcium chloride? *Ans.* 36.1 lb.

The Normal System.

Some of the more important determinations in water and sewage analysis are dependent upon the use of standard solutions. A standard solution is one which contains a known weight of the active substance dissolved in a definite volume of solution. Methods involving the use of such solutions are known as volumetric procedures since the quantitative result is obtained by measurement of volumes.

In the water and sewage laboratory, it is convenient to have solutions of certain definite strengths for use in the different determinations. Such solutions simplify the calculation of results, a decided advantage in routine analysis. They can best be prepared by diluting portions of stock solutions in such a manner as to give solutions of the desired strength.

The strength of a standard solution is usually expressed in terms of the normal system. A *normal* solution is one which contains one gram-equivalent weight of the active substance in one liter of solution. The *gram-equivalent* weight of a substance is the weight of that substance which is equivalent to one gram of hydrogen.

There are two general types of chemical reactions encountered in the volumetric determinations used for water and sewage analysis: (1) the simple neutralization or double-decomposition reactions, (2) the reactions involving oxidation and reduction.

The reactions of the first type involve a change in the position of the various atoms and groups making up the reacting substances. The following equations illustrate this type of reaction.

$$HCl + NaOH = NaCl + H_2O$$
$$H_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O$$

In a reaction of this type, the gram-equivalent weight of the compounds reacting is calculated by dividing the molecular weight of the compound by the number of replaceable hydrogen atoms or their equivalent in that compound.

$$\frac{\text{Molecular weight of a substance}}{\text{No. of replaceable hydrogen atoms or their equivalent}} = \text{gram-equivalent weight of the substance}$$

Thus the gram-equivalent weight of HCl is 36.5, since the molecular weight (36.5) is divided by 1, because there is one replaceable hydrogen. The gram-equivalent weight of H_2SO_4 is one-half its molecular weight, or 49, because there are two replaceable hydrogens. In NaOH one Na can be replaced by one H and is equivalent to one atom of hydrogen. Therefore, the gram-equivalent weight of NaOH is 40 (molecular weight divided by 1).

The second type of reaction, oxidation-reduction, is illustrated by the following equation:

$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 = 2MnSO_4 + K_2SO_4 + 10CO_2 + 8H_2O$$

A study of the equation will show that there is more involved than a simple rearrangement of atoms and groups. Mn, for instance, has a positive valence of seven in $KMnO_4$, but only two in $MnSO_4$. The Mn has lost five positive valences (has been reduced) and the $KMnO_4$ has a change in valence of five. Likewise, each C atom in $H_2C_2O_4$ has a valence of positive three and in CO_2 a valence of positive four. The compound $H_2C_2O_4$ has, therefore, a change in positive valence of two (one for each of the two C atoms).

The gram-equivalent weight of a compound entering into an oxidation-reduction reaction is equal to its molecular weight divided by the *change in valence* of that compound.

$$\frac{\text{Molecular weight}}{\text{Change in valence}} = \text{gram-equivalent weight}$$

The gram-equivalent weight of $KMnO_4$ is 31.6 (158 divided by 5) and of $H_2C_2O_4$ is 45 (90 divided by 2).

A number of reactions of these two types and the gram-equivalent weights of the compounds involved are given in Tables 4 and 5, pages 209 and 211.

The normality of a standard solution is the ratio of the weight in grams of the substance in one liter to the gram-equivalent weight.

$$\frac{\text{Weight in grams per liter}}{\text{Gram-equivalent weight}} = \text{normality (N)}$$

Solutions of equal normalities are equal in their reacting value, volume for volume. For example, a volume of 0.1N hydrochloric acid will react with the same volume of 0.1N sodium hydroxide.

The use of normalities simplifies the calculations necessary in obtaining the results of a volumetric analysis. For example, a sample of a solution of iodine is titrated with 0.1N sodium thiosulfate. Ten milliliters of the thiosulfate are required to react with the iodine. It can then be assumed that the amount of iodine present was that which would be contained in 10 ml. of a 0.1N iodine solution. The equivalent weight of iodine is 126.9 One liter of 0.1N iodine would contain 12.69 grams of iodine or 10 ml. would contain 0.1269 gram. Thus, the sample used contained 0.1269 gram of iodine.

Problems.

1. What is the hydrogen equivalent of Fe in ferric sulfate, in ferrous sulfate and in ferrous ammonium sulfate? *Ans.* 3, 2, 2.

2. What is the equivalent weight of potassium dichromate in the following reaction?

$$K_2Cr_2O_7 + 6KI + 7H_2SO_4 = 4K_2SO_4 + Cr_2(SO_4)_3 + 7H_2O + 3I_2$$

Ans. 49.04.

3. What is the normality of a solution of acetic acid (CH_3COOH) having 12.0064 gm. of acetic acid per liter? *Ans.* 0.2N.

4. If it requires 23.6 ml. of NaOH solution to neutralize 25 ml. of a 0.1N HCl solution, what is the normality of the NaOH solution?

Ans. 0.1059N.

STANDARD SOLUTIONS

Stock solutions should always be made stronger than those to be used in the various determinations. Strong solutions usually keep better than weak ones. In preparing the standard solutions for use in the various tests, the stronger solution may be diluted to give the desired normality. The portion of a stock solution required to make 1 liter of a desired normality may be calculated as follows:

$$\frac{1{,}000 \times \text{desired normality}}{\text{Normality of stock solution}} = \text{ml. of stock solution}$$

Stock standard solutions of some chemicals may be made by direct weight since the chemical can be obtained in a pure state. An example is anhydrous sodium carbonate. Others such as sulfuric acid and potassium permanganate must be standardized by titration against some pure chemical.

Stock standard acid solutions can be prepared by titrating against anhydrous sodium carbonate. The best grade of C.P. anhydrous sodium carbonate must be used. Methyl orange is used as an indicator.

The reaction involved in this titration is represented by the following equation:

$$\begin{array}{cccc} H_2SO_4 + Na_2CO_3 = & Na_2SO_4 + & H_2CO_3 \\ 98.08 \quad 105.99 & 142.05 & 62.02 \end{array}$$

The method for calculating the normality of the acid solution from the results of the titration can best be illustrated by a typical problem.

Calculations.

Weight of sodium carbonate used, 0.2138 gm.
Volume of sulfuric acid solution required for the titration, 37.1 ml.
Equivalent weight of $H_2SO_4 = 98.08/2 = 49.04$
Equivalent weight of $Na_2CO_3 = 105.99/2 = 53.00$

(1) 0.2138 gm. Na_2CO_3 is equivalent to $\frac{49.04}{53.00} \times 0.2138 = 0.1978$ gm. H_2SO_4

(2) 0.1978 gm. H_2SO_4 was in 37.1 ml. of solution

$$\frac{0.1978}{37.1} \times 1{,}000 = 5.331 \text{ gm. } H_2SO_4 \text{ in 1 liter of solution}$$

(3) $5.331/49.04 = 0.1087$, the normality of the acid solution

If the three steps above were placed in one equation, it would read as follows:

$$\frac{49.04 \times 0.2138 \times 1{,}000}{53.00 \times 37.1 \times 49.04} = 0.1087\text{N}$$

The general formula would, therefore, be:

$$\frac{\text{Weight of } Na_2CO_3 \times 1{,}000}{\text{Ml. of } H_2SO_4 \times 53.00} = \text{normality of acid solution}$$

The same method is used in the standardization of hydrochloric acid.

$$2HCl + Na_2CO_3 = 2NaCl + H_2CO_3$$

The same general type of formula given above may be used in calculating the results.

A stock standard solution of an alkali, such as sodium hydroxide, is usually prepared by titrating against a standard acid solution.

$$H_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O$$
$$HCl + NaOH = NaCl + H_2O$$

The normality of the sodium hydroxide solution is calculated as illustrated in the following example using the sulfuric acid, the strength of which was calculated above.

Calculations.

Volume of NaOH solution used = 25 ml.
Volume of H_2SO_4 solution used = 33.4 ml.
Normality of H_2SO_4 solution used = 0.1086

(1) 0.1086N H_2SO_4 contains 0.1086 × 49.04 = 5.326 gm. H_2SO_4 per liter or 5.326/1,000 = 0.005326 gm. per milliliter
(2) 33.4 × 0.005326 = 0.1779 gm. H_2SO_4 used in the titration
(3) The equivalent weight of NaOH is 40.05
The equivalent weight of H_2SO_4 is 49.04
0.1779 gm. H_2SO_4 is equivalent to

$$\frac{40.05}{49.04} \times 0.1779 = 0.1450 \text{ gm. NaOH}$$

(4) 0.1450 gm. NaOH in 25 ml. = 5.800 gm. NaOH per liter
(5) 5.800/40.05 = 0.1450N, the normality of the NaOH

Combining the above five steps:

$$\frac{0.1086 \times 49.04 \times 33.4 \times 40.05 \times 1{,}000}{1{,}000 \times 49.04 \times 25 \times 40.05} = 0.1450\text{N}$$

The general formula derived above is:

$$\frac{\text{Normality of acid} \times \text{ml. of acid}}{\text{Ml. of alkali}} = \text{normality of alkali}$$

This is a general formula which is applicable to all titrations involving two solutions when using the normal system. The formula may be restated as follows:

Ml. of one solution × its normality = ml. of the other solution × its normality

Sodium hydroxide solution must be made up with carbon dioxide-free water because of the reaction of the NaOH with the CO_2.

$$2NaOH + CO_2 = Na_2CO_3 + H_2O$$

The solution deteriorates if not kept free from carbon dioxide.

Stock standard sodium thiosulfate may be prepared of sufficient accuracy for some determinations, such as dissolved oxygen, by direct weight of the unweathered crystals. The equivalent weight of crystallized sodium thiosulfate ($Na_2S_2O_3 \cdot 5H_2O$) is 248.12. Therefore, 248.12 grams of the crystals is required for 1 liter of 1N sodium thiosulfate.

For a more accurate standardization or for restandardization of a previously prepared solution, titration against a weighed amount of potassium biniodate ($KIO_3 \cdot HIO_3$) may be conveniently used.

The reactions involved are as follows:

(1) $2KIO_3 \cdot HIO_3 + 20KI + 11H_2SO_4 = 11K_2SO_4 + 12H_2O + 12I_2$

(2) $24Na_2S_2O_3 + 12I_2 = 12Na_2S_4O_6 + 24NaI$

Adding equations (1) and (2) gives equation (3) as follows:

(3) $2KIO_3 \cdot HIO_3 + 20KI + 11H_2SO_4 + 24Na_2S_2O_3 = 11K_2SO_4 + 12Na_2S_4O_6 + 20NaI + 4NaI + 12H_2O$

The 20 KI on the left side of equation (3) balances the 20 NaI on the right side. The four iodine atoms of the biniodate appear on the right side in the 4 NaI. These four biniodate iodine atoms have a total valence of 20 positive. They are changed to four negative, or a total change of 24 for the two molecules of biniodate. Therefore 12 is the change in valence of each biniodate molecule. The equivalent weight of biniodate is its molecular weight divided by 12 or 390/12 = 32.50. The general formula for calculating the normality of the thiosulfate from the weight of biniodate and the titration results may be derived in the same manner as used for the acid solution previously described. This general formula is:

$$\frac{\text{Weight of biniodate used (gm.)} \times 1{,}000}{\text{Ml. of thiosulfate} \times 32.5} = \text{normality of sodium thiosulfate}$$

A standard solution of sodium oxalate may be prepared by a direct weight of the pure crystals ($Na_2C_2O_4$). The equivalent weight is the molecular weight divided by 2 or 134.00/2 = 67.00. Solutions of sodium oxalate are not stable and should be prepared fresh about every 2 weeks.

Standard solutions of potassium permanganate are prepared by titration against sodium oxalate solutions. The equations for the reactions are:

$$Na_2C_2O_4 + H_2SO_4 = H_2C_2O_4 + Na_2SO_4$$

$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 = 2MnSO_4 + K_2SO_4 + 10CO_2 + 8H_2O$$

This reaction has been given previously as an oxidation-reduction reaction.

The derivation of the formula for calculating the normality of the permanganate solution from the results of the titration is the same as that used for the standardization of sodium hydroxide previously discussed, except that the equivalent weights of potassium permanganate (31.60) and sodium oxalate (67.00) are used. The general formula is:

$$\frac{\text{Normality of oxalate} \times \text{ml. of oxalate}}{\text{Ml. of permanganate}} = \text{normality of permanganate}$$

Problems.

1. Calculate the normality of a hydrochloric acid solution standardized as follows:

Weight of anhydrous sodium carbonate = 1.003 gm.
Volume of hydrochloric acid required for titration = 21.6 ml.
Ans. 0.0876N.

2. What weight of oxalic acid is required to make 1 liter of 0.35N oxalic acid solution? *Ans.* 15.7528 gm.

ALKALINITY AND ACIDITY

Alkalinity.

Procedure, pages 8, 56 and 90.

There are three kinds of alkalinity, hydroxide (OH), normal carbonate (CO_3), bicarbonate (HCO_3). Normal carbonate is

also called monocarbonate. In order to distinguish between the kinds of alkalinity present in a sample and to determine the quantities of each, a titration is made with a standard acid using two indicators successively. The indicators used are phenolphthalein and methyl orange. Phenolphthalein ($C_{20}H_{14}O_4$) gives a pink color only in the presence of hydroxide or normal carbonate. The change from pink to colorless occurs at a pH value of 8.3. Methyl orange [$(CH_3)_2NC_6H_4N{:}NC_6H_4SO_3Na$] is yellow in the presence of any of the three types of alkalinity and red in the presence of acid. The change in color occurs at a pH value of approximately 4.4.

Normal carbonate alkalinity may be present with either hydroxide or bicarbonate alkalinity, but hydroxide and bicarbonate cannot be present together in the same sample. If there is phenolphthalein alkalinity in a sample, it is due to the presence of either hydroxide or normal carbonate or both. If there is methyl orange alkalinity present, it is due to any one of the three alkalinities, or hydroxide and normal carbonate together, or normal carbonate and bicarbonate together.

The following equations illustrate the reactions occurring when each of the three types of alkalinity is titrated with an acid.

1. Hydroxide:

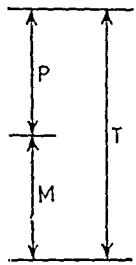
$$2NaOH + H_2SO_4 = Na_2SO_4 + 2H_2O$$

2. Normal carbonate:

$$2Na_2CO_3 + H_2SO_4 = 2NaHCO_3 + Na_2SO_4$$
$$2NaHCO_3 + H_2SO_4 = Na_2SO_4 + 2H_2CO_3$$

3. Bicarbonate:

$$2NaHCO_3 + H_2SO_4 = Na_2SO_4 + 2H_2CO_3$$



There are five alkalinity conditions possible in a sample: (1) hydroxide alone, (2) hydroxide and normal carbonate, (3) normal carbonate alone, (4) normal carbonate and bicarbonate, (5) bicarbonate alone. These five conditions may be distinguished and the quantities determined from the results of acid titrations (ordinarily the sample is titrated with 0.02N sulfuric acid) by the method given below in which:

P represents the alkalinity as shown by phenolphthalein.

T represents the total alkalinity or that shown by methyl orange.

$M = T - P$ or the additional alkalinity shown by methyl orange beyond that shown by phenolphthalein.

Condition 1 is fulfilled when $P = T$ or M = zero, because P indicates either hydroxide or normal carbonate, but normal carbonate would give a positive value for M because the phenolphthalein end point occurs when one-half of the normal carbonate reaction is completed. If M = zero, there can be no normal carbonate present.

Condition 2 is fulfilled when P is greater than $\frac{1}{2}T$, but less than T, or M is greater than zero. Since M is greater than zero, there must be some normal carbonate alkalinity present. M measures one-half of the normal carbonate, therefore, the normal carbonate equals $2M = 2(T - P)$. But the condition states that P is greater than $\frac{1}{2}T$ or greater than $2M$; therefore, there must be some hydroxide alkalinity present. The hydroxide alkalinity equals the total alkalinity minus the normal carbonate alkalinity or $T - 2(T - P) = 2P - T$.

Condition 3 is fulfilled when $P = \frac{1}{2}T$ or $P = M$. Since M represents one-half of the normal carbonate and since $P = M$, then P must represent the other half and only normal carbonate can be present. Normal carbonate $= 2P = T$.

Condition 4 is fulfilled when P is less than $\frac{1}{2}T$ (M is greater than P). M can be greater than P only when bicarbonate is present in addition to normal carbonate. This precludes hydroxide. The alkalinity represented by P is due to one-half of the normal carbonate. Then $2P$ = normal carbonate and the bicarbonate $= T - 2P$.

Condition 5 is fulfilled when P = zero. In this case there can be no hydroxide or normal carbonate. The alkalinity is all bicarbonate $= T$.

If the results of the titration of a 100-ml. sample with 0.02N H_2SO_4 are substituted in the correct condition above and these results multiplied by 10, the values obtained will be in p.p.m. of the specific alkalinity in terms of $CaCO_3$. (Alkalinities are usually expressed in terms of $CaCO_3$.) The factor 10 is used because 1 ml. of 0.02N sulfuric acid is equal to 1 mg. of $CaCO_3$.

If a 100-ml. sample is used, 1 ml. of acid would represent 1 mg. of $CaCO_3$ per 100 ml., or 10 mg. per liter, or 10 p.p.m.

Calculations.

Why is 1 ml. of 0.02N sulfuric acid equal to 1 mg. of $CaCO_3$?

The equivalent weight of H_2SO_4 = 49.04

The equivalent weight of $CaCO_3$ = 50.05

One liter of 0.02N H_2SO_4 contains $49.04 \times 0.02 = 0.9808$ gm. of H_2SO_4 or 1 ml. contains 0.0009808 gm. or 0.9808 mg. of H_2SO_4.

One ml. of 0.02N H_2SO_4 is then equivalent to

$$\frac{50.05}{49.04} \times 0.9808 = 1.00 \text{ mg. of } CaCO_3$$

Problem.

1. Calculate the alkalinities (as $CaCO_3$) in each of the following water samples. Titrations were made with 0.02N sulfuric acid and 100 ml. of sample.

Sample	Ml. of acid using phenolphthalein	Additional ml. of acid using methyl orange
A	none	46.2
B	5.6	20.7
C	16.4	8.0
D	13.8	none

Ans. A, bicarbonate 462 p.p.m.
B, carbonate 112 p.p.m., bicarbonate 151 p.p.m.
C, hydroxide 84 p.p.m., carbonate 160 p.p.m.
D, hydroxide 138 p.p.m.

Acidity.

Procedure, pages 9, 57 and 92.

Acidity in water and sewage is usually due to carbon dioxide, mineral acids and hydrolized salts. Most waters are considered alkaline although they may contain free carbon dioxide (CO_2) which in the presence of water forms carbonic acid (H_2CO_3). For this reason, many waters contain both acidity and alkalinity, in which case the acidity can only be due to carbon dioxide. Carbon dioxide is determined by titration with standard sodium hydroxide using phenolphthalein as an indicator:

$$CO_2 + NaOH = NaHCO_3$$

Some waters may be affected by mine drainage resulting in the presence of sulfuric acid. Industrial wastes may also contribute various acids to water and sewage. These acids are also determined by titration with standard sodium hydroxide.

Hydrogen-ion Concentration (pH).

Procedure, pages 10 and 71.

The pH determination is used in the control of water and sewage-treatment processes. In order to interpret properly the results of a pH determination, it is essential to have a knowledge of the meaning of the term and an idea of the value of the readings in terms of acidity and alkalinity. pH is defined as the "logarithm (base 10) of the reciprocal of the hydrogen-ion concentration." The "concentration" of the hydrogen ion is expressed in molecular weights (mols) per liter. Since the molecular weight of the hydrogen ion is 1, a solution containing 1 gram of hydrogen ion per liter would have a concentration of 1. The term "concentration of hydrogen ion" is written thus $[H^+]$. The brackets mean "concentration" and the H^+ means "hydrogen ion."

Water (H_2O) breaks down (dissociates) to a very slight extent into electrically charged particles called "ions," thus $HOH = H^+ + OH^-$. The H ion carries a positive charge and the OH a negative. There is an equilibrium established for any given condition when the rate of dissociation of the H_2O molecule into H^+ and OH^- is equal to the rate of association of H^+ and OH^- into the molecule H_2O. This may be written thus,

$$[H_2O] \rightleftarrows [H^+] + [OH^-] \qquad \text{or} \qquad \frac{[H^+][OH^-]}{[H_2O]} = K$$

This last equation means that at equilibrium the product of the concentration of H^+ ion $\times$ OH^- ion divided by the concentration of the undissociated H_2O molecule is equal to a constant (K). The value of $[H_2O]$ is so large in comparison to the dissociated ions that it can be given a constant value (w). The equation then reads $[H^+][OH^-] = Kw$.

Kw has been proven by experiment to be 1/100,000,000,000,000, or $1/10^{14}$. The reciprocal of a number with a positive exponent is equal to the number with the same negative exponent,

hence $Kw = 10^{-14}$. In pure water, then, $[H^+] = 10^{-7}$ and $[OH^-] = 10^{-7}$. The logarithm of $10^{-7} = -7$ but in its use to indicate the pH the negative sign is omitted, therefore, in pure water $[H^+]$ = pH 7. Such a solution is neutral and has no excess of either H^+ or OH^-.

In any solution, in order to maintain the equilibrium $[H^+][OH^-] = 10^{-14}$, if the $[H]^+$ is increased the $[OH^-]$ must be decreased accordingly. For example, if $[H^+]$ is increased to 10^{-4} then $[OH^-]$ must be decreased to 10^{-10} ($10^{-4} \times 10^{-10} = 10^{-14}$). The pH in this case would be 4. An excess of H^+ is indicated by a pH below 7 and the solution is said to be acid. pH values above 7 indicate an excess of OH^- or an alkaline condition.

As the pH values extend farther from pH 7 the acidity or alkalinity increases. The increases are not in direct proportion to the pH values but are logarithmic functions of those values. That is, a solution with a pH of 5 is ten times as acid as one with pH 6, and one with pH 4 is 100 times as acid as one with a pH of 6.

pH and titratable acidity are not the same except in cases where the acid or acid salts in the solution are completely ionized. In water and sewage the compounds are present in such minute amounts that the pH and titratable acid are more nearly the same than in stronger solutions. There are many factors, however, affecting the ionization of compounds and hence the pH, but these can not be discussed here.

For completely ionized solutions, the following comparison can be made between the normalities of acid solutions and pH. These values are based on the fact that in a completely ionized normal acid solution there is 1 gram of hydrogen ion per liter.

pH	Normality of Completely Ionized Acid
3.0	0.001
4.0	0.0001
5.0	0.00001
6.0	0.000001

Figure 1 (reproduced by permission of Buck, Seifert & Jost, consulting engineers) shows the pH values for various concentrations of carbon dioxide and bicarbonate alkalinity. This avoids the need of running the CO_2 test when pH and alkalinity have been determined.

Two general methods are available for the determination of pH, namely, the electrometric and colorimetric. There are several types of electrometric apparatus. Glass electrodes are commonly used. Electrometric methods are convenient for use, especially for solutions in which color changes are difficult or impossible to determine. Electrometric methods are largely replacing colorimetric methods in water and sewage analyses.

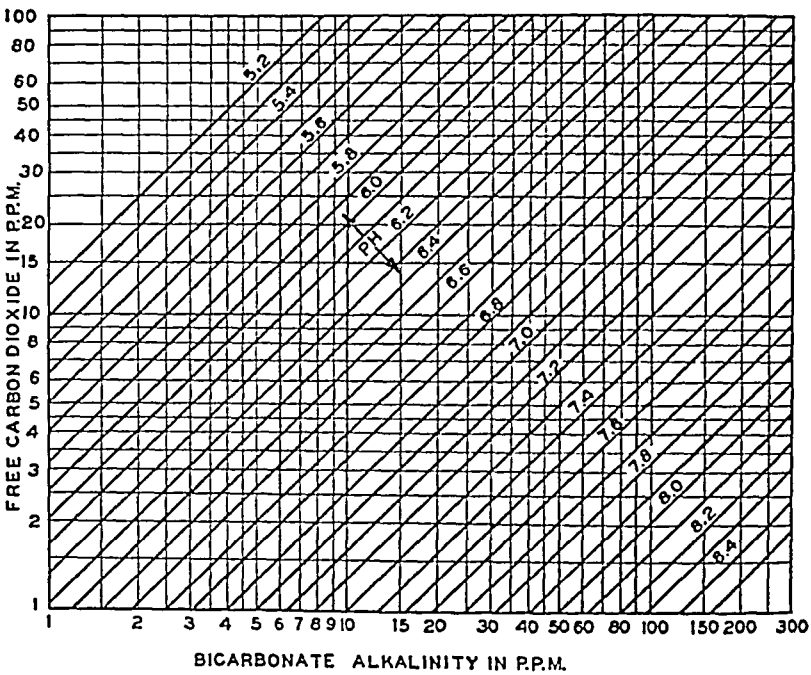


FIG. 1.

Colorimetric methods make use of the changes in color which certain dyes undergo under various changes in hydrogen-ion concentration. This change in color is not complete with a very small change in pH but extends over a comparatively wide range of pH. Indicators have certain definite pH ranges for color change and the indicator selected should be governed by the pH of the sample tested. The accuracy of the indicator is greatest near the center of its range and decreases as the limits are approached. The following are some indicators and the pH ranges covered by them.

Indicator	pH Range
Benzo yellow	2.4 to 4.0
Bromphenol blue	3.0 to 4.6
Bromcresol green	3.8 to 5.4
Chlorphenol red	5.2 to 6.8
Bromthymol blue	6.0 to 7.6
Phenol red	6.8 to 8.4
Cresol red	7.2 to 8.8
Thymol blue	8.0 to 9.6
Tolyl red	10.0 to 11.6

COAGULANTS AND COAGULATION

Coagulants are used in nearly all water treatment plants and in some sewage treatment plants. They are used (1) to remove natural suspended and colloidal material, (2) to remove materials which do not settle readily in chemical treatment processes, (3) to assist in filtration by forming mats on sand filters and (4) to assist in the vacuum filtration of sewage sludge.

A coagulant reacts with the natural alkalinity in solution in the liquid treated, or with other added chemicals, to form an insoluble, flocculant precipitate. The precipitate clarifies the liquid by coagulating, absorbing and entraining suspended and colloidal material. It may also remove colors and gases in solution. A portion of the precipitate formed in water filtration plants is carried through the sedimentation basins to be deposited on the filter beds where it further assists in the clarification of the water and removal of color-, odor- and taste-producing compounds.

The chemicals most commonly used as coagulants in water treatment are aluminum sulfate (alum), iron sulfate (copperas), ferric sulfate, iron chloride and sodium aluminate. Their use results in the formation of the hydroxides or aluminates which are the effective coagulating agents. In some waters containing magnesium, use is made of the magnesium hydroxide formed upon the addition of lime.

Alum is the most widely used coagulant. Commercial alum contains combinations of $Al_2(SO_4)_3 \cdot 18H_2O$ and $Al_2(SO_4)_3 \cdot 9H_2O$. Alum reacts with the natural alkalinity of the water or, if the alkalinity is insufficient, with the added alkalinity in the form of lime [$Ca(OH)_2$] or soda ash (Na_2CO_3), producing a precipitate which is usually considered to be aluminum hydroxide. Ordi-

narily there is sufficient natural alkalinity present to react with the coagulant. The reactions follow:

1. Alum and natural alkalinity:

$$Al_2(SO_4)_3 \cdot XH_2O + 3Ca(HCO_3)_2 = 2Al(OH)_3 + 3CaSO_4 + XH_2O + 6CO_2$$

2. Alum and added alkalinity:
   a. Alum and lime:

$$Al_2(SO_4)_3 \cdot XH_2O + 3Ca(OH)_2 = 2Al(OH)_3 + 3CaSO_4 + XH_2O$$

   b. Alum and soda ash:

$$Al_2(SO_4)_3 \cdot XH_2O + 3Na_2CO_3 = 2Al(OH)_3 + 3Na_2SO_4 + 3CO_2 + XH_2O$$

The amount of alum or other chemicals cannot be exactly determined from the above equations as there are many variables affecting the reactions. Actual plant experience is necessary with each particular water. Generally the amount of alum used is from 0.3 to 3 grains per gallon although some waters require less than 0.2 and others as high as 10 grains per gallon. The theoretical amounts of alkalinities, as required by the above equations, for each grain per gallon of alum, as $Al_2(SO_4)_3 \cdot 18H_2O$, used are:

1. Natural alkalinity as $CaCO_3$..... 0.4505 grain per gallon
2. *a*. Lime, as CaO..... 0.2524 grain per gallon
   Lime, as $Ca(OH)_2$..... 0.3336 grain per gallon
   b. Soda ash, as Na_2CO_3..... 0.4773 grain per gallon

Ferrous sulfate ($FeSO_4 \cdot 7H_2O$) requires the addition of lime. The reaction is as follows:

$$FeSO_4 \cdot 7H_2O + Ca(OH)_2 = Fe(OH)_2 + CaSO_4 + 7H_2O$$

The dissolved oxygen present in the water oxidizes ferrous hydroxide to ferric hydroxide as follows:

$$4Fe(OH)_2 + O_2 + 2H_2O = 4Fe(OH)_3$$

The ferric hydroxide is an effective coagulant. The use of this iron and lime treatment process requires careful regulation of the proper amounts of each chemical. As with alum, the exact amount of the chemicals, lime and iron sulfate, required cannot be determined except by actual plant experience. According to the above equation, the theoretical amount of lime [$Ca(OH)_2$]

required for each grain per gallon of ferrous sulfate ($FeSO_4 \cdot 7H_2O$) is 0.2665 grain per gallon. Ferrous sulfate is used mainly in conjunction with sufficient lime to provide softening rather than with only enough lime for straight coagulation.

Ferric sulfate [$Fe_2(SO_4)_3$] may be used with natural or added alkalinity. The reactions are, respectively, as follows:

(1) $Fe_2(SO_4)_3 + 3Ca(HCO_3)_2 = 3CaSO_4 + 2Fe(OH)_3 + 6CO_2$
(2) $Fe_2(SO_4)_3 + 3Ca(OH)_2 = 3CaSO_4 + 2Fe(OH)_3$

Ferrisul and Ferri-Floc are commercial names for ferric sulfate.

Ferric chloride ($FeCl_3$) reacts (1) with the natural alkalinity present in water or (2) with added alkalinity, as $Ca(OH)_2$, as follows:

(1) $2FeCl_3 + 3Ca(HCO_3)_2 = 2Fe(OH)_3 + 3CaCl_2 + 6CO_2$
(2) $2FeCl_3 + 3Ca(OH)_2 = 2Fe(OH)_3 + 3CaCl_2$

The effective coagulant is the ferric hydroxide as is the case when ferrous sulfate is used. For each grain per gallon of ferric chloride ($FeCl_3$) used, the theoretical amount of alkalinity required according to above equation is:

(1) Natural alkalinity, as $CaCO_3$ = 0.9255 grain per gallon
(2) Added alkalinity, as $Ca(OH)_2$ = 0.6852 grain per gallon

Sodium aluminate ($Na_2Al_2O_4$) has been successfully used as a coagulant for water treatment. It reacts with calcium and magnesium salts in water to form the aluminates of the metals. Both calcium and magnesium aluminates are effective as coagulants. The reactions follow:

$$Ca(HCO_3)_2 + Na_2Al_2O_4 = CaAl_2O_4 + Na_2CO_3 + CO_2 + H_2O$$
$$Mg(HCO_3)_2 + Na_2Al_2O_4 = MgAl_2O_4 + Na_2CO_3 + CO_2 + H_2O$$
$$CaSO_4 + Na_2Al_2O_4 = CaAl_2O_4 + Na_2SO_4$$
$$MgSO_4 + Na_2Al_2O_4 = MgAl_2O_4 + Na_2SO_4$$
$$CaCl_2 + Na_2Al_2O_4 = CaAl_2O_4 + 2NaCl$$
$$MgCl_2 + Na_2Al_2O_4 = MgAl_2O_4 + 2NaCl$$

When both lime and sodium aluminate are added to water, the following typical reactions take place:

(1) $Ca(OH)_2 + Na_2Al_2O_4 = CaAl_2O_4 + 2NaOH$
(2) $Ca(HCO_3)_2 + 2NaOH = CaCO_3 + Na_2CO_3 + 2H_2O$
(3) $CaSO_4 + Na_2CO_3 = CaCO_3 + Na_2SO_4$

The aluminate of calcium in equation (1) is the effective coagulant. Equations (2) and (3) may be considered as softening reactions incidental to and accompanying the use of lime and sodium aluminate for coagulation. If softening is desired, it can be most economically secured by adding lime [$Ca(OH)_2$] and soda ash (Na_2CO_3) for the removal of the bicarbonates and sulfates, respectively.

Chemicals used in treatment of sewage are lime, ferrous and ferric sulfates and ferric chloride. The reactions involved are the same as those already given for water treatment. Care must be used not to add too much lime, a treatment which results in a caustic sewage and consequent solution of suspended organic matter.

Problems.

1. Calculate the weight of alum required, in pounds per million gallons, to give a dose of 0.4 g.p.g. What is the application in p.p.m.?

Ans. 57.16 lb., 6.85 p.p.m.

2. What weight of natural bicarbonate alkalinity, as $CaCO_3$, will be required to react with the alum in the above problem?

Ans. 25.72 lb. per million gallons

3. How much lime, as $Ca(OH)_2$, will be theoretically required to furnish alkalinity for an application of 2 lb. of copperas per 1,000 gal. in the treatment of a trade waste? *Ans.* 0.53 lb.

WATER HARDNESS AND SOFTENING

Hardness of Water.

Hardness of water is caused principally by the elements calcium and magnesium and sometimes by iron and aluminum. Iron and aluminum are seldom present, in waters usable as water supplies, in sufficient amounts to have much significance in the hardness determinations, although they are often present in sufficient quantity to cause other undesirable effects on the water supply. For this reason iron and aluminum will not be further discussed in connection with hardness and it will be assumed that hardness is caused entirely by calcium and magnesium.

Most of the calcium and magnesium is present in natural waters as bicarbonates, sulfates and sometimes as chlorides and nitrates. Hardness-producing substances react with soaps, forming insoluble compounds before a lather is produced. They

are thus a measure of the soap-consuming power of a water. They also deposit scale in boilers and hot-water heating systems.

Temporary hardness is that removed by boiling. Permanent hardness is that remaining after boiling. Temporary hardness is caused principally by the presence of bicarbonates of calcium and magnesium. Permanent hardness is due mostly to calcium sulfate, which is precipitated at temperatures above 300°F. Carbonate hardness is due to the presence of calcium and magnesium normal carbonates and bicarbonates. Noncarbonate hardness includes the calcium and magnesium sulfates, chlorides and nitrates. Sulfates are often the only noncarbonate hardness compounds present. Compounds causing permanent hardness are often termed "incrustants."

Hardness is always expressed in terms of calcium carbonate ($CaCO_3$). Methods of analysis and calculations used in the laboratory give results in terms of $CaCO_3$. Alkalinity is also expressed in the same terms. Thus a report showing a water to have a hardness of 100 p.p.m. does not signify just what compounds cause the hardness but only that the hardness is equivalent to that produced by 100 p.p.m. of $CaCO_3$.

Total hardness is most accurately found by determining the amounts of calcium and magnesium (and sometimes iron and aluminum) by a gravimetric analysis and by calculating their equivalent values in terms of calcium carbonate ($CaCO_3$). An approximate determination of total hardness may be made with a soap solution of known strength. Total hardness may also be obtained by the soda-reagent method. This method is to be preferred to the soap method.

Carbonate hardness is found by calculation from the results of the normal carbonate and bicarbonate alkalinity determinations. If the normal carbonate and bicarbonate alkalinity, expressed in terms of $CaCO_3$, is greater than the total hardness, normal carbonates or bicarbonates of sodium or potassium are present. These compounds do not cause hardness and, in this case, the carbonate hardness would be equal to the total hardness. If the sum of the normal carbonate and bicarbonate alkalinities is equal to the total hardness, the carbonate hardness is also equal to the total hardness. If the sum of the normal carbonate and bicarbonate alkalinities is less than the total hardness, this

sum is equal to the carbonate hardness and the difference between this sum and the total hardness is the noncarbonate hardness.

The methods for the gravimetric determination of calcium and magnesium, and equations involved in these determinations, are given under Mineral Analysis, pages 160 to 161. In the calculations under the methods for making these determinations the results are obtained in p.p.m. of the elements. In order to change these to terms of hardness it is necessary to convert them to their respective equivalents of $CaCO_3$ and add the results. The general formulas used for this purpose are obtained from a comparison of the equivalent weights.

Example.

Equivalent weight of $CaCO_3$ = 50.05
Equivalent weight of Ca = 20.04
Equivalent weight of Mg = 12.16

(1) P.p.m. Ca × 50.04/20.04 = p.p.m. Ca as $CaCO_3$
(2) P.p.m. Mg × 50.04/12.16 = p.p.m. Mg as $CaCO_3$
(3) P.p.m. Ca as $CaCO_3$ + p.p.m. Mg as $CaCO_3$ = total hardness as $CaCO_3$

The soap test is a rapid method for the determination of total hardness. The results obtained are only approximate. The end point is difficult to obtain and is often unsatisfactory. Its advantage is in its rapidity and as a comparison with the results obtained by other methods.

Total hardness by the soda-reagent method is also a rapid method for the determination of total hardness. Sulfuric acid is first added to decompose the normal carbonates and bicarbonates. The sample is boiled to remove carbon dioxide. The addition of the soda reagent, a mixture of sodium hydroxide and sodium carbonate, precipitates the calcium and magnesium. The sodium hydroxide precipitates the magnesium as magnesium hydroxide and sodium carbonate precipitates the calcium as calcium carbonate. These precipitates are filtered off and the filtrate titrated with 0.02N sulfuric acid. A distilled-water sample is treated in the same manner. The difference between the acid used for the sample and that used for the distilled water represents the hardness of the sample. The reactions are as follows:

Addition of acid to remove the carbonate hardness:

$$CaCO_3 + H_2SO_4 = CaSO_4 + H_2CO_3$$
$$Ca(HCO_3)_2 + H_2SO_4 = CaSO_4 + 2H_2CO_3$$
$$MgCO_3 + H_2SO_4 = MgSO_4 + H_2CO_3$$
$$Mg(HCO_3)_2 + H_2SO_4 = MgSO_4 + 2H_2CO_3$$

Precipitation of calcium and magnesium with soda reagent:

$$CaSO_4 + Na_2CO_3 = CaCO_3 + Na_2SO_4$$
$$MgSO_4 + 2NaOH = Mg(OH)_2 + Na_2SO_4$$

Titrating the excess soda reagent with sulfuric acid:

$$2NaOH + Na_2CO_3 + 2H_2SO_4 = 2Na_2SO_4 + H_2CO_3 + 2H_2O$$

The solubility of the $CaCO_3$, formed after addition of the soda reagent, introduces a slight error. This compound is soluble to the extent of about 15 p.p.m. at ordinary temperatures. The soluble calcium carbonate will pass through the filter paper and react with the acid during the titration, giving a low result. For this reason the gravimetric method of determining calcium and magnesium hardness is more accurate. However, the results obtained by the soda-reagent method are sufficiently accurate for the ordinary water treatment plant control.

Water acquires its hardness from contact with mineral-bearing materials in the earth's surface. Rainfall, after reaching the earth, takes up carbon dioxide and organic acids from the soil and loses some or all of its dissolved oxygen. Limestone is dissolved by water containing carbon dioxide, resulting in the formation of calcium bicarbonate, a soluble compound.

$$CaCO_3 + H_2O + CO_2 = Ca(HCO_3)_2$$

The sulfates and chlorides of calcium and magnesium are comparatively soluble and the sulfates, in particular, are found in appreciable quantities in many water supplies.

Water Softening.

Water having less than 50 to 75 p.p.m. of hardness is generally considered as sufficiently soft for the ordinary uses of a public water supply. Water having 75 to 150 p.p.m. of hardness may be considered as moderately hard but still is not sufficiently hard

to interfere seriously with its use for most purposes or to cause much public demand for water softening. Hardness above 150 p.p.m. is noticed by most persons and, if the hardness is above 200 p.p.m., many homes will be provided with household softeners or cisterns.

Water for municipal purposes is most commonly softened by the lime-soda process. Lime, as $Ca(OH)_2$, is used to remove the magnesium and the carbonate hardness. The lime is purchased either in the hydrate form [$Ca(OH)_2$] or as the oxide (CaO), in which latter case it is slaked at the plant site to produce the hydrate. Soda ash is used to remove the noncarbonate hardness.

The equations expressing the reactions occurring when lime is added to remove the calcium and magnesium bicarbonates are as follows:

(1) $Ca(HCO_3)_2 + Ca(OH)_2 = 2CaCO_3 + 2H_2O$
(2) $Mg(HCO_3)_2 + Ca(OH)_2 = MgCO_3 + CaCO_3 + 2H_2O$
(3) $MgCO_3 + Ca(OH)_2 = Mg(OH)_2 + CaCO_3$

The first equation shows the reaction of calcium (as calcium bicarbonate) with lime, resulting in precipitation of the normal carbonate ($CaCO_3$). The removal is not complete because $CaCO_3$ is soluble to the extent of about 15 p.p.m. The second and third equations show the removal of magnesium by precipitating as magnesium hydroxide. If insufficient lime is added, $MgCO_3$, a soluble compound, will be formed, as shown in equation 2. The use of sufficient lime results in the formation of $Mg(OH)_2$, an insoluble compound, as shown in equation 3. It will be noticed that more lime is required for the removal of magnesium bicarbonate than for calcium bicarbonate hardness.

Lime also reacts with any free carbon dioxide present as follows:

(4) $CO_2 + Ca(OH)_2 = CaCO_3 + H_2O$

The removal of calcium noncarbonate hardness requires the addition of soda ash (Na_2CO_3), and the removal of magnesium noncarbonate hardness requires the addition of both lime and soda ash. The following equations show the reactions involved:

(5) $CaSO_4 + Na_2CO_3 = CaCO_3 + Na_2SO_4$
(6) $MgSO_4 + Ca(OH)_2 = Mg(OH)_2 + CaSO_4$
$CaSO_4 + Na_2CO_3 = CaCO_3 + Na_2SO_4$
(7) $CaCl_2 + Na_2CO_3 = CaCO_3 + 2NaCl$
(8) $MgCl_2 + Ca(OH)_2 = Mg(OH)_2 + CaCl_2$
$CaCl_2 + Na_2CO_3 = CaCO_3 + 2NaCl$

In order to determine the amount of chemicals required in the lime and soda ash process of water softening, it is necessary to make certain chemical tests. Methods of performing these tests are given elsewhere in this book. The tests needed are (1) free carbon dioxide (CO_2), (2) alkalinity, (3) noncarbonate (incrustant) hardness, (4) magnesium.

From the equations previously given, the theoretical amounts of lime and soda ash have been calculated and are given below for use with the results of these tests. Actual amounts of chemicals can only be determined by plant operation. Bottle experiments, paralleling the plant conditions, are useful in determining the amounts of chemicals needed.

The total lime required is equivalent to the sum of the carbon dioxide, bicarbonate alkalinity and magnesium content. The theoretical amount of lime, as CaO, required for free CO_2 is:

P.p.m. free $CO_2 \times 0.01062$ = lb. CaO required per 1,000 gal. of water

P.p.m. free $CO_2 \times 0.0743$ = grains CaO required per gallon of water

Lime (CaO) required for bicarbonate alkalinity, as $CaCO_3$, is:

P.p.m. bicarbonate alkalinity $\times 0.00467$ = lb. CaO required per 1,000 gal. of water

P.p.m. bicarbonate alkalinity $\times 0.0327$ = grains CaO required per gallon of water

The bicarbonate alkalinity, as $CaCO_3$, may be expressed as half-bound CO_2 by multiplying it by 0.44. This value of CO_2 may then be added to the free CO_2 and the amount of lime required, for both CO_2 and bicarbonate alkalinity per 1,000 gal. of water, obtained by multiplying the sum by 0.01062.

Additional lime (CaO) required for magnesium, as Mg, is:

P.p.m. Mg $\times 0.01922$ = lb. CaO required per 1,000 gal. of water

P.p.m. Mg × 0.1345 = grains CaO required per gallon of water

Since commercial quicklime is not pure CaO, the amount of lime (CaO) required by above calculations should be multiplied by 100/per cent CaO in the quicklime used. If calcium hydrate [$Ca(OH)_2$] is used instead of quicklime (CaO), the amount of commercial calcium hydrate would be obtained from the above calculations by multiplying by

$$\frac{100}{\text{Per cent CaO in the lime hydrate used}}$$

High-calcium quicklime averages about 90 per cent CaO and the hydrate about 65 per cent CaO. The factors to use for obtaining the theoretical amounts of pure CaO or $Ca(OH)_2$ are summarized in the table below. They should be multiplied by 100/per cent purity to obtain the amount of commercial chemical required.

Item determined, in p.p.m.	Factors by which to multiply values in first column to obtain required amounts of pure			
	Hydrate of lime, $Ca(OH)_2$		Quicklime, CaO	
	Lb. per 1,000 gal.	Grains per gal.	Lb. per 1,000 gal.	Grains per gal.
CO_2 (as CO_2)	0.01403	0.09821	0.01062	0.0743
Bicarbonate alkalinity as $CaCO_3$	0.006169	0.04318	0.00467	0.0327
Mg (as Mg)	0.02539	0.1777	0.01922	0.1345

Soda ash (Na_2CO_3) required to remove the noncarbonate hardness, as $CaCO_3$, is:

P.p.m. noncarbonate hardness × 0.00883 = lb. Na_2CO_3 required per 1,000 gal. of water

P.p.m. noncarbonate hardness × 0.0618 = grains of Na_2CO_3 required per gallon of water

Illustrative Problem.

A water shows the following characteristics. Determine the amounts of lime and soda ash theoretically required to remove all the hardness.

Free carbon dioxide (CO_2) = 30 p.p.m., as CO_2
Alkalinity:
with phenolphthalein = 0 p.p.m., as $CaCO_3$
with methyl orange = 270 p.p.m., as $CaCO_3$
Magnesium = 40 p.p.m., as Mg
Noncarbonate hardness = 60 p.p.m., as $CaCO_3$

Calculations.

(Using factors from above table.)
Free CO_2:
$30 \times 0.01062 = 0.32$ lb. of CaO per 1,000 gal. of water
Alkalinity:
$270 \times 0.00467 = 1.26$ lb. of CaO per 1,000 gal. of water
Magnesium:
$40 \times 0.01922 = 0.77$ lb. of CaO per 1,000 gal. of water
Total = 2.35 lb.

If quicklime, containing 90 per cent water-soluble CaO, were used, the amount of quicklime required would be:

$$\frac{2.35}{0.90} = 2.61 \text{ lb. per 1,000 gal. of water}$$

If lime hydrate, containing 65 per cent CaO, were used, the amount of hydrate required would be:

$$\frac{2.35}{0.65} = 3.62 \text{ lb. per 1,000 gal. of water}$$

Noncarbonate-hardness removal would require the following amount of soda ash: $60 \times 0.00883 = 0.530$ lb. of Na_2CO_3 per 1,000 gal. of water. If the soda ash were 97 per cent pure Na_2CO_3, the amount of soda ash required would be 0.530/0.97 = 0.547 lb. of soda ash per 1,000 gal. of water.

If alum was also used (to remove raw water turbidity, to assist in settling of precipitates or to assist in filtration), additional amounts of lime and soda ash would be needed as shown in the following equations:

(1) $Al_2(SO_4)_3 \cdot 18H_2O + 3Ca(OH)_2 = 2Al(OH)_3 + 3CaSO_4 + 18H_2O$
(2) $3CaSO_4 + 3Na_2CO_3 = 3CaCO_3 + 3Na_2SO_4$

The amounts of lime, as $Ca(OH)_2$, and soda ash (Na_2CO_3) required, as calculated from the above equations are:

Lime [$Ca(OH)_2$] = 0.3335 for each part of alum [$Al_2(SO_4)_3 \cdot 18H_2O$]

Soda ash (Na_2CO_3) = 0.4776 for each part of alum [$Al_2(SO_4)_3 \cdot 18H_2O$]

Problems.

1. Calculate the weight of quicklime (90 per cent CaO) and soda ash (98 per cent Na_2CO_3) necessary to soften a water having the following analysis. Express the results in lb. per 1,000 gal.

Note.—This result will be theoretical, since it is not possible to soften completely with lime and soda ash.

Free CO_2	15 p.p.m.
Calcium	70 p.p.m.
Magnesium	25 p.p.m.
Bicarbonate alkalinity	200 p.p.m. as $CaCO_3$

Ans. 1.75 lb. lime, 0.70 lb. soda ash

2. Using the above values calculate the temporary and permanent (noncarbonate) hardness of the water.

Ans. 200 p.p.m. temporary, 77.7 p.p.m. permanent

3. Assuming noncarbonate hardness to be entirely due to calcium sulfate, calculate the SO_4 content of the water. *Ans.* 74.6 p.p.m. SO_4

CHEMISTRY OF MINERAL ANALYSIS

Magnesium, Volumetric Method.

Procedure, page 14.

The volumetric determination of magnesium consists of adding equal volumes of limewater to distilled water and the sample, respectively. The magnesium in the sample is precipitated as the insoluble hydroxide and filtered.

$$MgSO_4 + Ca(OH)_2 = Mg(OH)_2 + CaSO_4$$

The amount of lime required to precipitate the magnesium in the sample is determined by titration of the distilled water and the filtered sample with 0.02N sulfuric acid.

$$Ca(OH)_2 + H_2SO_4 = CaSO_4 + 2H_2O$$

The difference between the milliliters of acid required for the distilled water and the milliliters required for the sample is used to calculate the amount of lime employed in removing the

magnesium. This difference × 9.72 gives the p.p.m. magnesium. The factor 9.72 is obtained as follows:

Calculations.

Equivalent weight of H_2SO_4 = 49.04
Equivalent weight of Mg = 12.16

(1) One milliliter of 0.02N H_2SO_4 contains
0.02 × 49.04/1,000 = 0.000980S gm. or 0.980S mg. of H_2SO_4

(2) 0.980S mg. H_2SO_4 is equivalent to

$$12.16/49.04 \times 0.980S = 0.243 \text{ mg. of Mg}$$

(3) In the instructions for the determination of magnesium 25 ml. of the sample was used. (100 ml. of the sample was diluted to 200 ml. and then 50 ml. of the diluted sample used.) Then

$$\frac{0.243 \times 1{,}000}{25} = 9.72 \text{ mg. of Mg per liter or 9.72 p.p.m. per milliliter of 0.02N acid used}$$

Sulfates, Volumetric Method.

Procedure, pages 14 and 93.

Ferric iron reacts with benzidine hydrochloride to give low results in the sulfate determination. Ferrous iron does not react in this manner. Hydroxylamine hydrochloride is added to reduce the iron to the ferrous state should it be present in sufficient quantities to interfere greatly with the determination.

Benzidine hydrochloride reacts with sulfates in a hydrochloric acid solution to form a slightly soluble compound of benzidine and sulfuric acid

$$CaSO_4 + C_{12}H_8(NH_2)_2 \cdot 2HCl = C_{12}H_8(NH_2)_2 \cdot H_2SO_4 + CaCl_2$$

This compound is filtered and washed entirely free of excess hydrochloric acid. The amount of sulfuric acid in the compound is then determined by titration with standard sodium hydroxide.

$$C_{12}H_8(NH_2)_2 \cdot H_2SO_4 + 2NaOH = Na_2SO_4 + C_{12}H_8(NH_2)_2 \cdot 2H_2O$$

Iron and Aluminum Oxides.

Procedure, page 18.

Concentrated hydrochloric acid is added to change the iron and aluminum oxides in the residue to the soluble chlorides.

$$6HCl + Fe_2O_3 = 2FeCl_3 + 3H_2O$$
$$6HCl + Al_2O_3 = 2AlCl_3 + 3H_2O$$

Bromine water is added to insure the oxidation of the iron to the ferric state. Ammonium chloride is added to prevent the formation of insoluble magnesium hydroxide when ammonium hydroxide is added. This addition of the ammonium ion decreases the hydroxide-ion concentration to a point where there is not sufficient to precipitate the magnesium hydroxide.

Iron and aluminum hydroxides are much less soluble than magnesium hydroxide and are precipitated.

$$FeCl_3 + 3NH_4OH = Fe(OH)_3 + 3NH_4Cl$$
$$AlCl_3 + 3NH_4OH = Al(OH)_3 + 3NH_4Cl$$

The combined precipitates are ignited and weighed as the oxides.

$$2Fe(OH)_3 = Fe_2O_3 + 3H_2O$$
$$2Al(OH)_3 = Al_2O_3 + 3H_2O$$

Calcium.

Procedure, page 19.

Calcium is precipitated from the water sample by the addition of ammonium oxalate after silica and iron and aluminum have been removed.

$$Ca(OH)_2 + (NH_4)_2C_2O_4 = CaC_2O_4 + 2NH_4OH$$

The calcium oxalate precipitate, after filtering, may be either ignited and weighed as the oxide (CaO)

$$CaC_2O_4 = CaO + CO_2 + CO$$

or may be titrated with potassium permanganate.

$$CaC_2O_4 + H_2SO_4 = H_2C_2O_4 + CaSO_4$$
$$2KMnO_4 + 5H_2C_2O_4 + 3H_2SO_4 = 2MnSO_4 + K_2SO_4 + 10CO_2 + 8H_2O$$

In the first case the weight of calcium in the sample is calculated from the weight of the CaO by using the equivalent weights thus:

The equivalent weight of Ca is 20.04 and of CaO is 28.04. The weight of Ca in CaO is 20.04/28.04 × weight of CaO.

In the second case the potassium permanganate is made of such a strength that, when a 250-ml. sample is used, the milliliters of permanganate × 10 = p.p.m. calcium. Each ml. of permanganate, therefore, must be equivalent to 2.5 mg. of calcium. The weight of permanganate necessary to prepare 1 liter of such a solution is calculated by the use of the equivalent weights.

Calculations.

Equivalent weight of $KMnO_4$ = 31.60
Equivalent weight of Ca = 20.04

$\frac{31.60}{20.04} \times 2.5 = 3.94$ mg. of $KMnO_4$ per milliliter required = 3.94 gm. per liter

The normality of such a solution is calculated thus:

$$\frac{3.94}{31.6} = 0.1246N$$

Magnesium, Gravimetric Method.

Procedure, page 20.

Silica, iron and aluminum and calcium must be removed from the sample before magnesium is determined. The magnesium is precipitated as magnesium ammonium phosphate ($MgNH_4PO_4$).

$$MgCl_2 + NH_4OH + Na_2HPO_4 = MgNH_4PO_4 + 2NaCl + H_2O$$

This precipitate is filtered and ignited to magnesium pyrophosphate ($Mg_2P_2O_7$), in which form it is weighed.

$$2MgNH_4PO_4 = Mg_2P_2O_7 + 2NH_3 + H_2O$$

In order to calculate the magnesium in the pyrophosphate the equivalent weights are again used.

Calculations.

Equivalent weight of $Mg_2P_2O_7$ = 55.67
Equivalent weight of Mg = 12.16

(1) The weight of $Mg_2P_2O_7 \times 12.16/55.67$ = gm. of Mg in sample
(2) To convert this weight to p.p.m.

Gm. × 1,000 = mg. per sample

If a 250-ml. sample is used,

Mg. per sample × 4 = mg. per liter or p.p.m.

Combining the above steps, the general formula is obtained:

Weight of $Mg_2P_2O_7$ (gm.) × 873.6 = p.p.m. Mg

Sulfate, Gravimetric Method.

Procedure, page 21.

Sulfates are determined by precipitating with barium chloride and filtering, igniting and weighing the precipitate, barium sulfate.

$$CaSO_4 + BaCl_2 = BaSO_4 + CaCl_2$$

To calculate the weight of sulfate in the barium sulfate precipitate, the equivalent weights are used.

Calculations.

Equivalent weight of $BaSO_4$ = 116.71
Equivalent weight of SO_4 = 48.03

(1) Weight of $BaSO_4$ (gm.) × 48.03/116.71 = gm. of SO_4 in sample
(2) To convert this weight to p.p.m. Gm. × 1,000 = mg. per sample. If a 250-ml. sample is used, the mg. per sample × 4 = mg. per liter or p.p.m.

Combining the above steps gives a general formula: Weight of $BaSO_4$ (gm.) × 1646.1 = p.p.m. SO_4

Sodium and Potassium, Gravimetric Method.

Procedure, page 22.

Sodium and potassium are converted into their chlorides and weighed as such. These chlorides are very soluble and, in order to obtain them free from other compounds, all of the other metals and negative radicals must be removed from the sample. When this is accomplished, the residue of the sodium and potassium chlorides, after the evaporation of the water, is weighed and the results expressed in terms of sodium.

In order to convert the weight of sodium and potassium chloride to sodium, it is necessary to assume that the entire weight is sodium chloride and use the equivalent weights as follows:

Calculations.

Equivalent weight of NaCl = 58.46
Equivalent weight of Na = 23.00

(1) Weight of NaCl (gm.) × 23.00/58.46 = gm. Na in sample
(2) Gm. Na in sample × 1,000 = mg. per sample
(3) If 250-ml. sample is used, mg. per sample × 4 = mg. per liter or p.p.m.

Combining the above steps the general formula is obtained.

$$\text{Gm. NaCl} \times 1573.6 = \text{p.p.m. Na}$$

Potassium.

Procedure, page 23.

In order to distinguish between sodium and potassium in a sample, the potassium is precipitated from the combined chlorides with platinic chloride.

$$2KCl + H_2SO_4 = K_2SO_4 + 2HCl$$
$$K_2SO_4 + H_2PtCl_6 = K_2PtCl_6 + H_2SO_4$$

The potassium chloroplatinate precipitate (K_2PtCl_6) is filtered, ignited and weighed. In order to obtain the weight of potassium chloride from the weight of the precipitate the equivalent weights are used.

Calculations.

Equivalent weight of K_2PtCl_6 = 243.10
Equivalent weight of KCl = 74.56
Equivalent weight of K = 39.10

$$\text{Weight of } K_2PtCl_6 \text{ (gm.)} \times \frac{74.56}{243.10} = \text{gm. KCl in sample}$$

$$\text{Gm. KCl in sample} \times \frac{39.10}{74.56} = \text{gm. K in sample}$$

Chloride, Volumetric Method.

Procedure, pages 15, 68 and 94.

In this procedure, the chloride of the sample is determined by titration with a standard silver nitrate solution in the presence of potassium chromate indicator. The chloride is precipitated a silver chloride (a white precipitate).

$$NaCl + AgNO_3 = AgCl + NaNO_3$$

Hydrogen sulfide interferes with the reaction, precipitating black silver sulfide.

$$H_2S + 2AgNO_3 = Ag_2S + 2HNO_3$$

Calculations.

1 ml. of $AgNO_3$ solution = 0.5 mg. chloride radical

(Ml. of $AgNO_3$ solution used -0.2) $\times$ 0.5 = mg. chloride in sample

$$\frac{\text{Ml. } AgNO_3 \text{ solution} \times 0.5 \times 1{,}000}{\text{Ml. of sample}} = \text{mg. chloride per liter} = \text{p.p.m.}$$

A correction of 0.2 ml. is made for the excess silver nitrate required to produce a noticeable amount of red precipitate.

The end point of the titration is reached when a red precipitate of silver chromate (Ag_2CrO_4) first appears owing to the reaction of a slight excess of silver nitrate with the potassium chromate.

$$K_2CrO_4 + 2AgNO_3 = Ag_2CrO_4 + 2KNO_3$$

Iodide.

Procedure, page 16.

This iodide test has been used in an extensive study of the effect of the iodine content of water supplies on the goiter incident in Michigan. Since the iodine content of waters is usually extremely low, a very large sample is necessary. The major portion of the mineral matter is removed by evaporation and extraction with alcohol. The iodides are soluble in alcohol while many of the other salts are not. The determination depends upon the color developed by iodine when dissolved in carbon disulfide.

Fluoride.

Procedure, page 17.

The fluoride test is made in connection with the effect of fluorine in water supplies on the prevalence of dental caries. The test depends upon the color developed by fluorine in combination with zirconium-alizarin reagent. This test was originally developed by J. M. Sanchis.

Silica.

Procedure, page 17.

Silica occurs in water in the form of very finely divided clay particles and of silicates in colloidal solution. These compounds upon being acidified produce the insoluble silicic acids.

$$Na_2SiO_3 + 2HCl = H_2SiO_3 + 2NaCl$$

The acids upon drying and ignition are dehydrated, producing silica.

$$H_2SiO_3 = SiO_2 + H_2O$$

Sodium by Difference.

Procedure, page 26.

The quantitative determination of sodium is complicated and time consuming. This element is often present in such small amounts in water that an accurate test is difficult. For ordinary purposes, a sufficiently accurate estimate of the quantity of sodium present may be obtained by calculation from the difference between the positive and negative ions as determined by the mineral analysis.

Any complete mineral analysis of water must show a balance between the reacting or combining weights of the positive and negative elements or radicals. The positive elements in the usual water are calcium, magnesium, iron, aluminum and sodium. The negative elements or radicals are bicarbonate, carbonate, sulfate and chloride. Others are usually present in such small quantities as to be of no significance for ordinary purposes.

The weights of the elements or radicals in any particular water react in proportion to their respective equivalent weights (see pages 209 to 210). The following proportion, for instance, may be written for the elements A, B and C:

$$\frac{\text{Weight of } A}{\text{Equivalent weight of } A} = \frac{\text{weight of } B}{\text{equivalent weight of } B} = \frac{\text{weight of } C}{\text{equivalent weight of } C}$$

The value $\frac{\text{weight of } A}{\text{equivalent eight of } A}$ is known as the "reacting value" of A, etc. For convenience in making the calculations,

the actual weights are multiplied by the reciprocals of the respective equivalent weights. For example: The equivalent weight of calcium is 20.04. Then, the reacting value would be

$$\text{Weight of calcium} \times \frac{1}{20.04}$$

or

$$\text{Weight of calcium} \times 0.0499$$

The sum of the reacting values of all positive ions must be equal to the sum of the reacting values of all negative ions. Since in the complete mineral analysis, all significant positive and negative ions are determined except sodium, that element may be determined by difference.

Total (or Residual) Iron.

Procedure, page 27.

Iron may be present in two forms, namely the reduced form (ferrous) and the fully oxidized form (ferric). Ferric iron is seldom found in true solution in natural waters, unless they are highly acid, because of the formation of insoluble ferric oxide hydrates. These hydrates occur in colloidal solution in many cases.

Ferrous salts are more likely to be found in true solution, although they are easily oxidized to the ferric state and precipitated in alkaline waters as the hydrated oxides. Ferrous iron has a positive valence of two while ferric has three.

Ferric iron is determined by producing a red-colored iron compound, ferric thiocyanate, by the addition of potassium thiocyanate.

$$FeCl_3 + 3KCNS = Fe(CNS)_3 + 3KCl$$

The red color produced is compared with color standards.

Ferrous iron is also determined colorimetrically by producing a blue compound, known as Turnbull's blue, by the addition of potassium ferricyanate.

$$3FeCl_2 + 2K_3Fe(CN)_6 = Fe_3[Fe(CN)_6]_2 + 6KCl$$

The quantity of ferrous iron is determined by comparison with the color produced by known amounts of ferrous compounds treated in a similar manner.

Both of the standard iron solutions are prepared from ferrous ammonium sulfate crystals [$FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$]. The weight of this compound necessary to prepare a solution containing 0.1 mg. of iron per milliliter is calculated as follows:

Calculations.

Equivalent weight of ferrous ammonium sulfate crystals = 196.07
Equivalent weight of ferrous iron = 27.92
(1) 0.1 mg. per milliliter = 0.1 gm. per liter
(2) $0.1 \times 196.07/27.92 = 0.7023$ gm. ferrous ammonium sulfate crystals per liter.

Manganese, Colorimetric.

Procedure, page 29.

Manganese is determined by oxidation of the manganous compounds to permanganate with ammonium persulfate and a comparison of the color produced with standard manganous sulfate solutions treated in a similar manner.

$$2MnSO_4 + 5(NH_4)_2S_2O_8 + 8H_2O + 10HNO_3 = 2HMnO_4 + 12H_2SO_4 + 10NH_4NO_3.$$

The standard manganous sulfate solution is prepared by reducing, with oxalic acid, the necessary amount of potassium permanganate to make 1 liter of solution so that 1 ml. = 0.1 mg. Mn. The amount of permanganate necessary is determined as follows:

Calculations.

Molecular weight of $KMnO_4$ = 158.03
Atomic weight of Mn = 54.93
(1) 0.1 mg. per milliliter = 0.1 gm. per liter
(2) $0.1 \times 158.03/54.93 = 0.2877$ gm. $KMnO_4$

Residual Alum, Colorimetric Method.

Procedure, page 30.

The logwood test for the presence of residual alum in filtered water has been used as a qualitative test for many years. The alizarin red S test given in this manual has many advantages over the logwood test, one being its adaptation as a quantitative procedure.

The test makes use of the formation of a colored compound by the reaction of aluminum with the dye. This color is com-

pared with that of a standard alum solution treated in the same manner. The stock alum solution is prepared by dissolving an approximate amount of aluminum sulfate crystals [$Al_2(SO_4)_3 \cdot 18H_2O$] in water and standardizing by a gravimetric analysis for aluminum. The method used is similar to that used for the iron and aluminum oxides determination. This stock solution is then diluted so that 1 ml. = 0.01 mg. Al_2O_3.

Problems.

1. A 250-ml. water sample was tested for silica according to method on page 17. The gain in weight in the last step of the test was 0.0025 gm. Compute p.p.m. of silica (SiO_2). *Ans.* 10 p.p.m. SiO_2.

2. A 250-ml. water sample was tested for iron and aluminum oxides, calcium and magnesium according to methods on pages 18 to 20.

   a. The increase in weight in the last step of the iron and aluminum oxides test was 0.0010 gm. Compute the p.p.m. of combined Fe_2O_3 and Al_2O_3.

   b. 9.0 ml. of potassium permanganate solution was used in the last step in the calcium test. Compute p.p.m. of calcium.

   c. A gain in weight of 0.0303 gm. was obtained in the last step of the magnesium test. Compute p.p.m. of magnesium.

   Ans. a. 4.0 p.p.m. $Fe_2O_3 + Al_2O_3$
   b. 88.0 p.p.m. Ca
   c. 26.4 p.p.m. Mg

3. A 250-ml. water sample was tested for sulfates according to method on page 21. The gain in weight in the last step of the test was 0.0120 gm. Compute the p.p.m. of sulfates (SO_4). *Ans.* 19.8 p.p.m. SO_4

4. The tests in problems 1, 2 and 3 above were all run on the same water. Its alkalinity to phenolphthalein was zero and to methyl orange 340 p.p.m., as $CaCO_3$. The iron was 1.6 p.p.m. and the chloride (Cl) was 6.5 p.p.m. Compute the p.p.m. sodium and potassium in terms of Na, by method given on page 26. *Ans.* 16.1 p.p.m. Na and K, as Na

CHLORINE DETERMINATION

Chlorine Demand.

Procedure, page 67.

Chlorination of water and sewage for sterilization and of sewage for odor control and reduction of biochemical oxygen demand is being practiced in many treatment plants. To be effective for sterilization, sufficient chlorine must be added to satisfy the chlorine demand and to leave residual chlorine after a contact period.

There is a number of methods used for the chlorine-demand determination. The one given in this manual is simple and sufficiently accurate for the majority of purposes. In this method a series of samples are treated with varying amounts of chlorine. After standing for 10 min. they are tested for residual chlorine. The sample with the least amount of added chlorine which shows a residual is used in calculating the chlorine demand. The starch-iodide test for residual chlorine is used.

Residual Chlorine.

Procedure, pages 30, 31, 67 and 68.

Residual chlorine is determined by either the orthotolidine or starch-iodine test. Residual chlorine by the orthotolidine method is subject to interference by nitrites and iron, causing high results or even indicating a chlorine content where none is present. The test, however, is in general use and, if the procedure given in this manual is carefully followed, will give satisfactory results. Chlorine and certain other oxidizing agents produce a yellow-colored compound with orthotolidine. This compound is probably the result of the oxidation of one of the amino (NH_2) groups of the hydrochloric acid salt of orthotolidine.

$$2H_2O + 2Cl_2 = 4HCl + O_2$$

$$NH_2{\cdot}CH_3{\cdot}C_6H_3{\cdot}C_6H_3{\cdot}CH_3{\cdot}NH_2{\cdot}HCl + O_2 = NO{\cdot}CH_3{\cdot}C_6H_3{\cdot}C_6H_3{\cdot}CH_3{\cdot}NH_2{\cdot}HCl + H_2O$$

Residual chlorine by the starch-iodide test depends upon the liberation of iodine from potassium iodide by free chlorine.

$$2KI + Cl_2 = I_2 + 2KCl$$

The presence of iodine is shown by the formation of a blue color upon the addition of starch.

Chlorine Break Point.

Procedure, page 31.

The residual chlorine in a chlorinated water supply is not rigidly proportional to the amount of chlorine added to the water, nor does it always increase with additional chlorine added. The usual amounts of chlorine necessary for sterilization of water supplies are only from 0.1 to 0.5 p.p.m., although for a water

high in organic matter or turbidity the amount of chlorine required may be several p.p.m.

Usually a water shows a continual rise in the chlorine residual until several p.p.m. of chlorine is added and then a definite decrease in residual chlorine covering a restricted range of increased chlorine dosages. The point at which the residual chlorine starts to decrease is called the break point. The point at which the break point occurs varies widely with different waters and in some waters is barely perceptible. The decreasing chlorine-residual range is of short duration and is followed by a rise in residual chlorine nearly proportional to the rate of chlorine application. This latter rise in residual chlorine starts after the chlorine demand of the water has been completely satisfied.

The significance of the break point in water treatment lies in the facts that chlorine may be applied at high rates for sterilization and that at or near this point tastes and odors in most water supplies disappear with nonobjectionable residual of chlorine.

A flash test for chlorine break point is given on page 32. (This test was developed by Paul C. Laux and J. B. Nickel.) The use of the orthotolidine solution of low acid content (66A), instead of the usual orthotolidine solution (66), produces no color until the amount used is in excess of that required for the chlorine break point. The color developed is blue but if the water has a pH above about 7.9, the blue color will change immediately to yellow. For waters of very high pH only the yellow color is obtained.

NITROGEN DETERMINATIONS

Organic Nitrogen.

Procedure, page 64.

The organic nitrogen determination is a measure of the nitrogen in the form of proteins or intermediate decomposition products. The ammonia nitrogen is first removed by boiling from a slightly alkaline solution. Ammonia can be volatilized from an alkaline medium but not from a strong acid.

After the removal of ammonia, concentrated sulfuric acid is added and the water distilled. The heating is continued until the organic compounds have been completely decomposed by

the hot acid, at which time the nitrogen will have been entirely converted to ammonium sulfate [$(NH_4)_2SO_4$]. The ammonia is liberated by making the solution alkaline with sodium hydroxide.

$$(NH_4)_2SO_4 + 2NaOH = Na_2SO_4 + 2NH_3 + 2H_2O$$

The solution is then distilled and the distillate containing the ammonia collected and analyzed for ammonia nitrogen.

Ammonia Nitrogen.

Procedure, pages 35 and 62.

The ammonia nitrogen determination depends upon a comparison of colors produced when the sample and standards are treated with Nessler reagent. Nessler reagent ($2KI \cdot HgI_2$) reacts with the ammonia in alkaline solution giving a yellow-colored compound.

$$2(2KI \cdot HgI_2) + 2NH_3 + 3KOH = (NH_2)_2HgOHHgI + 7KI + 2H_2O$$

In order to free the solution from interfering substances it must be either clarified by coagulation with copper sulfate, $CuSO_4 + 2NaOH = Cu(OH)_2 + Na_2SO_4$, or by distillation.

Albuminoid Nitrogen.

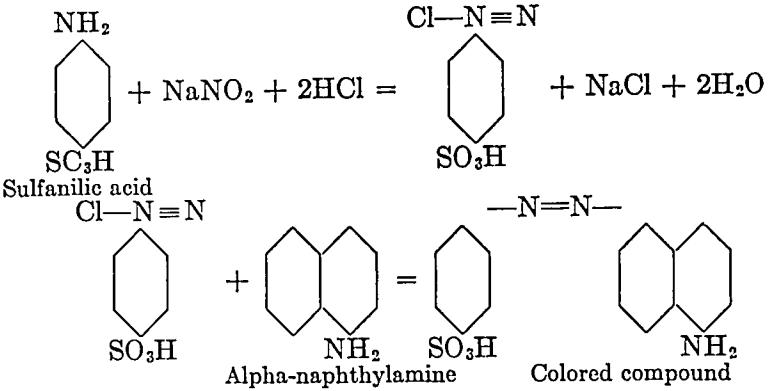
Procedure, page 63.

Sewage and water containing sewage pollution contain nitrogen in the form of organic compounds and urea. Part of this organic nitrogen is readily converted into ammonia by oxidizing with an alkaline solution of potassium permanganate. This part is known as "albuminoid nitrogen" because it is contained in albuminous compounds. Owing to natural oxidation and decomposition the nitrogenous compounds in sewage and water are broken down into a wide variety of intermediate organic compounds and ammonium salts. For this reason, the test for albuminoid nitrogen is not as indicative of the true nitrogen value of a sample as is the test for total nitrogen by the Kjeldahl method. The test is not used to a great extent except in cases where it is desirable to continue the accumulation of records.

Nitrite Nitrogen.

Procedure, pages 36 and 65.

The nitrite determination is made by comparing colors produced by treating the sample and standards with sulfanilic acid and alpha-naphthylamine hydrochloride. The resulting red color is due to the production of azobenzol-naphthylamine sulfonic acid. The reactions are represented by the following equations:



Nitrate Nitrogen.

Procedure, pages 37 and 66.

The nitrates are the final oxidation products of the organic nitrogen compounds. They may be determined in two ways: (1) By reducing them to ammonia with nascent hydrogen. The reduction takes place in a hydrochloric acid solution which immediately converts the ammonia to ammonium chloride.

$$NaNO_3 + 8H + 2HCl = NH_4Cl + NaCl + 3H_2O$$

The ammonia is then determined by the ammonia nitrogen method and converted to terms of nitrate nitrogen. (2) By the disulfonic acid method. Disulfonic acid is prepared by treating phenol with sulfuric acid.

$$C_6H_5OH + 2H_2SO_4 = C_6H_3(OH)(SO_3H)_2 + 2H_2O$$

When nitrates are treated with disulfonic acid and the resulting solution made alkaline with sodium hydroxide, a yellow com-

pound is produced. The compound is the sodium salt of picric acid formed by the nitration of the phenol.

$$C_6H_3(OH)(SO_3H)_2 + 3HNO_3 = C_6H_2(OH)(NO_2)_3 + 2H_2SO_4 + H_2O$$

The color produced by this compound in the sample is compared with potassium nitrate standards treated in a similar manner.

OXYGEN DETERMINATIONS

Oxygen Consumed.

Procedure, pages 40 and 60.

The oxygen-consumed determination is a measure of the amount of oxygen required to oxidize unstable materials in a sample by means of potassium permanganate in an acid solution. This test has been largely replaced by the biochemical-oxygen-demand determination since it does not give results which are comparable to those obtained from biological oxidation processes which occur in nature. The test has one advantage in that the results can be obtained in less than 1 hour while the biochemical test requires at least 5 days. Owing to the fact that potassium permanganate is selective in its reaction and attacks the carbonaceous and not the nitrogenous matter, the results will be different from those obtained by the biochemical oxygen-demand method.

Two standard solutions are required, potassium permanganate and ammonium oxalate. The preparation of stock standard solutions from which these standards can be prepared by dilution is discussed in Sec. II, pages 104 and 105. The standard solutions are prepared in such a manner that 1 ml. = 0.1 mg. of oxygen. (1 liter = 0.1 gram.) The normality of the solution to fulfill this condition may be determined as follows:

$$\text{Normality} = \frac{\text{gm. oxygen per liter}}{\text{equivalent weight of oxygen}} = \frac{0.1}{8.0} = 0.0125\text{N}$$

The stock standard solutions may be diluted to this normality using the quantity of stock solution as determined by the formula

$$\frac{1{,}000 \times 0.0125}{\text{Normality of stock solution}} = \text{ml. of stock solution for 1 liter of 0.0125N standard solution.}$$

The 0.0125N standard ammonium oxalate solution may also be prepared by direct weight according to the directions in Sec. II, page 118. The weight of ammonium oxalate [$(NH_4)_2C_2O_4 \cdot H_2O$] required to make 1 liter of a solution, 1 ml. of which is equal to 1 mg. oxygen, may be calculated as follows:

Equivalent weight of crystalline ammonium oxalate = 71.05.

A 0.0125N solution of ammonium oxalate will contain $71.05 \times 0.0125 = 0.8881$ gm. crystalline ammonium oxalate per liter.

Dissolved Oxygen, Winkler Method and Rideal-Stewart Modification.

Procedure, pages 38 and 39.

The Winkler method may be used for the determination of dissolved oxygen in the majority of cases encountered in the water and sewage plant laboratory. In some cases errors may be introduced by the presence of nitrites, iron salts and certain organic compounds. The effect of nitrites can be minimized by careful manipulation of the procedure, as will be shown later. This effect has been eliminated in the Winkler method as given in this text by the use of sodium azide. In cases where other interfering compounds are present in sufficient quantities to cause a significant error, the Rideal-Stewart modification must be used.

The reactions involved in the various steps of the Winkler method are represented by the following equations: Manganous sulfate reacts with the potassium hydroxide in the alkaline potassium iodide mixture to produce a white flocculent precipitate of manganous hydroxide:

$$MnSO_4 + 2KOH = Mn(OH)_2 + K_2SO_4$$

If the white precipitate is obtained, there was no dissolved oxygen in the sample and there is no need to proceed further. A brown precipitate shows that oxygen was present and reacted with the manganous hydroxide. The brown precipitate is manganic basic oxide:

$$2Mn(OH)_2 + O_2 = 2MnO(OH)_2$$

Upon the addition of the acid, this precipitate is dissolved, forming manganic sulfate:

$$MnO(OH)_2 + 2H_2SO_4 = Mn(SO_4)_2 + 3H_2O$$

There is an immediate reaction between this compound and the potassium iodide previously added, liberating iodine and resulting in the typical iodine coloration of the water:

$$Mn(SO_4)_2 + 2KI = MnSO_4 + K_2SO_4 + I_2$$

The quantity of iodine liberated by these reactions is equivalent to the quantity of oxygen present in the sample. The quantity of iodine is determined by titrating a portion of the solution with a standard solution of sodium thiosulfate:

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI$$

The thiosulfate is made of such a strength that 1 ml. = 0.2 mg. of oxygen. Such a solution would have a normality obtained as follows:

(1) 0.2 mg. per milliliter = 0.2 gm. per liter

(2) Equivalent weight of oxygen = 8.0

(3) $\frac{0.2}{8.0} = 0.025N$

The preparation of the stock standard solution from which this standard may be prepared was discussed on pages 104 and 105.

The error due to nitrites is introduced at the time the solution is made acid with sulfuric acid. In an acid medium, nitrites react with the potassium iodide, liberating iodine:

$$2KI + H_2SO_4 = 2HI + K_2SO_4$$
$$2HNO_2 + 2HI = 2H_2O + N_2O_2 + I_2$$

If the reaction was complete at this point, the error due to nitrites in most cases would not be significant. However, if the sample is allowed to stand exposed to the air, the oxygen which dissolves will react with the N_2O_2, again producing the nitrite:

$$2N_2O_2 + 2H_2O + O_2 = 4HNO_2$$

This will again liberate more iodine. Should this cycle be repeated a sufficient number of times, the error introduced would soon become very large. The continuous reaction can be minimized by an immediate and rapid titration of the sample after it is exposed to the air.

The reactions by which the effect of nitrites is eliminated by means of sodium azide (added in the alkaline potassium iodide) are as follows:

$$2NaN_3 + H_2SO_4 = 2HN_3 + Na_2SO_4$$
$$HNO_2 + HN_3 = N_2O + N_2 + H_2O$$

In the Rideal-Stewart modification, the nitrites, iron and organic matter are first oxidized by potassium permanganate and excess permanganate removed with potassium oxalate. Care must be taken not to add too great an excess of the oxalate or an error will be introduced in the final result.

Biochemical Oxygen Demand.

Procedure, pages 58 and 81.

The biochemical oxygen-demand determination is a measure of the oxygen required to oxidize the organic matter in a sample, through the agency of microscopic organisms (bacteria). The test consists of the determination of dissolved oxygen prior to and following a period of incubation at 20°C. The incubation period is usually 5 days. If the oxygen demand of the sample is greater than the available dissolved oxygen, a dilution is made. The amount of dilution depends upon the oxygen demand and must be such that an appreciable amount of dissolved oxygen (1.5 to 2.0 p.p.m. minimum) remains after the incubation period. For wastes or sewage having an unknown oxygen demand, it is necessary to make up a number of dilutions in order to be sure that one will meet the requirements.

There are a number of factors which influence the rate of oxidation of organic matter by bacteria and hence, the 5-day oxygen demand. The type of diluting water, pH value and bacterial content are the most important. The diluting water, prepared according to Sec. II, page 120, has been temporarily accepted as a standard diluting water. Some agencies, including the U.S. Public Health Service, have recommended the use of phosphate-buffered dilution waters. Various formulas have been suggested, most of which contain calcium and magnesium salts as well as phosphates and ammonium nitrate. These waters give higher B.O.D. values than those obtained with the bicarbonate water. Whether or not these values are nearer the

correct value is questionable. A standard water is the principal requirement, in order to standardize the procedure for making the B.O.D. test as much as possible. Until another water is generally accepted as a standard, the bicarbonate water is recommended. The optimum pH value is between 7.0 and 7.6 and under special conditions the pH may require adjustment prior to incubation. Sterilized sewage effluents or samples not containing a bacterial flora must be seeded with bacteria before incubation. It is apparent that directions cannot be given which will cover all special conditions encountered in making this test. The methods given will meet the majority of situations and the laboratory worker must be able to adopt modifications to meet special requirements.

Some samples of water and sewage have an immediate oxygen demand and it is sometimes desirable to know the quantity of this immediate demand. The demand is due to the presence of easily oxidizable substances such as hydrogen sulfide, iron, etc., substances which are encountered, for instance, in a septic sewage. Such a condition, of course, could only exist in samples entirely devoid of oxygen.

The immediate oxygen demand may be determined from two dissolved-oxygen determinations, one made on diluting water and the other on a dilution of the waste or sewage with the diluting water. This dilution must be of such strength that there will be an appreciable amount of oxygen remaining after mixing. The test on the dilution is made immediately following mixing. The initial demand is calculated as follows:

(1)

$$\frac{\text{P.p.m. D.O. in diluting water} \times \text{percentage of diluting water used}}{100}$$

= p.p.m. D.O. of the dilution before any immediate demand is exerted. Let this value = A

(2) P.p.m. D.O. of dilution after mixing = B

(3) Then immediate oxygen demand in p.p.m.

$$= (A - B) \times \frac{100}{\text{per cent sample in dilution}}$$

The 5-day biochemical oxygen demand does not represent the total demand of the sample for oxygen. Sometimes the

total demand is desired. This can be calculated from the following table which represents the percentage of the total oxygen demand satisfied at various periods of incubation of the sample.

The values given in this table apply to sewage, water and industrial wastes in a medium favorable for the growth and activity of the bacteria responsible for the oxidation of the organic matter. This must be borne in mind and allowance made for conditions which tend to interfere with the proper reaction. For instance, in the oxidation of some trade wastes there is a decided lag in the rate of oxidation owing to unfavorable conditions, or there may be a large initial or immediate demand which would not conform to the rate indicated.

The values given in the table are usually known as the "Relative stability numbers." Relative stability is also a measure

RATE OF BIOCHEMICAL OXIDATION

Period of incubation at 20°C., days	Percentage of demand satisfied	Period of incubation at 20°C., days	Percentage of demand satisfied
0.5	11	8.0	84
1.0	21	9.0	87
2.0	37	10.0	90
3.0	50	12.0	94
4.0	60	14.0	96
5.0	68	16.0	97
6.0	75	20.0	99
7.0	80		

of oxygen demand except that the values obtained are relative and not absolute. This test makes use of the fact that methylene blue is decolorized if no oxygen is available. A sample treated with methylene blue will remain colored as long as oxygen is present. At the time when the oxygen demand has removed the oxygen, a certain per cent of the demand is satisfied. This per cent is that given in the preceding table. For instance, if a sample remains colored for only 5 days there was originally sufficient oxygen in the sample to satisfy 68 per cent of the total oxygen demand.

To calculate the oxygen demand at any period from the biochemical oxygen-demand (B.O.D.) determination the following method may be used:

Let A = p.p.m. B.O.D. as determined.

B = per cent demand satisfied in the period used as obtained from preceding table.

C = per cent demand satisfied in the period desired as obtained from preceding table.

$\frac{A}{B} \times C$ = p.p.m. B.O.D. for period desired

Calculations.

The 5-day B.O.D. of a sample is found to be 140 p.p.m. What is the 10-day B.O.D.?

$A = 140$; $B = 68$; and $C = 90$

$\frac{140}{68} \times 90 = 185$ p.p.m. 10-day B.O.D.

Problems.

1. The B.O.D. of the influent and effluent of a sewage treatment plant are 195 and 106 p.p.m., respectively. Calculate the percentage removal. If the flow is 2.3 million gallons per day calculate the pounds of B.O.D. removed daily. *Ans.* 45.6 per cent, 170.7 lb.

2. A city with a population of 100,000 and a sewage flow of 125 gal. per capita per day is located on a stream with a rate of flow of 40 cu. ft. per sec. The B.O.D. of the sewage is 210 p.p.m. The dissolved-oxygen and B.O.D. content of the stream above the outfall sewer are 7.8 and 1.0 p.p.m., respectively. How many pounds per day of oxygen are available above the outfall? What is the total pounds of B.O.D. per day in the stream just below the outfall (assuming no oxidation has taken place)? Express this B.O.D. in p.p.m. *Ans.* 1680 lb., 22,110 lb., 69.2 p.p.m.

HYDROGEN SULFIDE

Procedure, page 69.

Hydrogen sulfide is determined by its reducing action on a standard iodine solution. The reaction is represented by the following equation:

$$H_2S + I_2 = 2HI + S$$

Since it is not feasible to titrate the sulfide directly, more than the required amount of the iodine solution is added and the excess determined by titration with standard sodium thiosulfate.

$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI$$

The iodine solution is prepared by dissolving iodine in a solution of potassium iodide. The presence of the iodide increases the solubility of the iodine. The solution is standardized against 0.025N sodium thiosulfate and adjusted to be equivalent to that solution.

The strength of the 0.025N iodine solution in terms of hydrogen sulfide is determined as follows:

Equivalent weight $H_2S = 17.05$

1 liter of 0.025N iodine solution $= 17.05 \times 0.025$
$= 0.426$ gm. H_2S

1 ml. of 0.025N iodine solution $= 0.426$ mg. H_2S

CHEMISTRY OF SLUDGE ANALYSIS

Fertilizer Value.

Procedure, page 73.

The value of sewage sludge as a fertilizer is low when compared with commercial fertilizers. Activated sludge has a higher nitrogen content than primary-tank digested sludge. These sludges have especially high values as soil conditioners. Their use as fertilizers and soil conditioners provides a satisfactory means of disposal.

The analysis consists of a determination of moisture, humus, phosphoric acid (P_2O_5), nitrogen and potash. The last is often omitted since the potash content is consistently low and the determination complicated and time consuming. The results of a fertilizer analysis are always based on the dry weight of the material, except the moisture determination which is based on the weight of the sample collected. As in all other determinations the accuracy of the results depends in a large measure on proper sampling. A discussion of sampling of sludge for fertilizer analysis is given on page 196.

Fertilizer values of commercial fertilizers are generally indicated by the percentages of nitrogen (N), phosphoric acid (P_2O_5) and potash (K_2O). Thus a 5–8–5 fertilizer contains 5 per cent nitrogen, 8 per cent phosphoric acid and 6 per cent potash. Commercial fertilizers vary widely in their total per cent of plant foods and in the ratios of the constituents. The following table gives values of plant-food constituents in manures, commercial fertilizers and digested sewage sludges.

FERTILIZER AND SLUDGE ANALYSES
Values in Per Cent (Dry Basis)

	Nitrogen (N)	Phosphoric acid (P_2O_5)	Potash (K_2O)
Poultry manure	2.3	0.9	1.1
Cow manure	2.8	0.8	2.5
Horse manure	2.6	1.1	2.4
Sheep manure	2.5	0.7	1.5
Activated-sludge sewage plant			
Digested sludge	3.5–5.5	1.5–3.0	0.2–0.3
Primary sewage plant			
Digested sludge	1.5–3.5	1.0–3.0	0.1–0.3
Commercial fertilizer:			
Low value	2	8	2
High value	4	16	8
Concentrated	4	24	12

Loss on Ignition (Humus).

Procedure, page 74.

The loss on ignition determination is not a true measure of the organic matter (humus) in a sludge because of the decomposition of inorganic salts, carbonates in particular, at the temperatures used. Almost every method calls for the ignition at "low red heat," which is a variable temperature depending upon the opinion of the worker as to what constitutes a "low red" color. The following table gives the temperatures and corresponding colors of heated bodies.

TEMPERATURE OF GLOWING BODIES

Color	Temperature	
	Degrees centigrade	Degrees Fahrenheit
First visible red	525	980
Dull red (low red)	600	1,112
Turning to cherry	800	1,472
Cherry proper	900	1,652
Bright cherry	1,000	1,892
Dull orange	1,100	2,012
Bright orange	1,200	2,192
White	1,300	2,632

Calcium carbonate is the principal carbonate in sewage sludge. This carbonate is either present originally in the sludge or is produced by the partial ignition of certain calcium organic salts. Although the temperature at which calcium carbonate decomposes is given as 825°C., yet there is evidence that partial decomposition takes place at much lower temperatures. This being the case, a variable partial decomposition may take place at 600°C. (low red heat) and a much greater decomposition at temperatures approaching 825°C.

The results of ignition at low red heat are subject to considerable error if the values are to be interpreted in terms of humus. This temperature is, however, required to assure the complete combustion of the carbon.

In order to obtain a more accurate value for humus in fertilizer, temperatures of ignition should be such as to insure a complete decomposition of the calcium carbonate and a correction then made. This procedure does not take into account other minor losses which may occur owing to the presence of other inorganic salts. Most of the other salts which might be present are soluble compounds and would be largely removed with the sludge liquor, sludge beds or filters. The amount of these salts present in most cases would be too small to have much significance.

The decomposition of calcium carbonate takes place according to the following equation:

$$\underset{100}{CaCO_3} = \underset{56}{CaO} + \underset{44}{CO_2}$$

The figures below the formulas in the above equation are the molecular weights. Thus, 44 parts in 100 or 44 per cent of the calcium carbonate is liberated as carbon dioxide gas during ignition.

The correction may be appreciable in some cases and of little significance in others. The calcium carbonate content of digested primary sludge varies from 5 to 12 per cent of the weight of the dried sludge.

In the determination of calcium the same procedure is used as that recommended for the determination of calcium in water. This method is discussed on page 160.

Phosphoric Acid Anhydride (P_2O_5).

Procedure, page 75.

Phosphates present are reported as their equivalents in terms of P_2O_5. In the phosphate determination, the organic matter in the dried sludge is first decomposed with sulfuric acid. After adjusting the solution to neutrality with nitric acid and ammonium hydroxide, the phosphate is precipitated with ammonium molybdate. The precipitate is ammonium phosphomolybdate $[(NH_4)_3PO_4 \cdot 12MoO_3]$.

This precipitate is dissolved in an excess of 0.3238N sodium hydroxide solution:

$$2(NH_4)_3PO_4 \cdot 12MoO_3 + 46NaOH = 2(NH_4)_2HPO_4 + (NH_4)_2MoO_4 + 23Na_2MoO_4 + 22H_2O$$

The excess of the 0.3238N hydroxide used is determined by titration with 0.3238N hydrochloric acid. The difference between the quantities used in the titration represents the amount of the hydroxide required to react in the above equation.

The 0.3238N acid and alkali are used because 1 ml. of alkali having this normality is equivalent to 1 mg. of P_2O_5, thus simplifying the calculations.

This value for the normality of the alkali can be obtained by consideration of the above equation. It will be seen that two molecules of ammonium phosphomolybdate react with 46 molecules of sodium hydroxide. The two molecules of ammonium phosphomolybdate contains two atoms of phosphorous, from which it follows that two atoms of phosphorus in the above equation are equivalent to 46 molecules of sodium hydroxide. Since this is true one molecule of P_2O_5 is equivalent to 46 molecules of NaOH and the equivalent weight of P_2O_5 is its molecular weight divided by 46, or $142.1/46 = 3.09$. A 1N solution of NaOH will, therefore, be equivalent to 3.09 gm. P_2O_5 per liter or 3.09 mg. per milliliter and a 0.3238N solution will be equivalent to $3.09 \times 0.3238 = 1$ mg. P_2O_5 per milliliter.

Nitrogen.

Procedure, page 76.

The nitrogen determination consists of first decomposing the sludge with a mixture of salicylic and sulfuric acids. The

salicylic acid is added to prevent the loss of nitrogen as nitrates since it has been found that nitrates are almost entirely decomposed by heating with sulfuric acid alone as follows:

$$2KNO_3 + H_2SO_4 = K_2SO_4 + 2HNO_3$$

The HNO_3 is volatile. Salicylic acid retains the nitrates in the form of the nitro compound:

$$HNO_3 + C_6H_4OHCOOH = C_6H_3OH(NO_3)COOH + H_2O$$
Nitro compound

Sodium thiosulfate is then added to reduce the nitro compound to an amino compound which is then broken down into ammonia.

$$Na_2S_2O_3 + H_2SO_4 = Na_2SO_4 + H_2SO_3 + S$$
$$6H_2SO_3 + 2C_6H_3OH(NO_3)COOH + 2H_2O = 6H_2SO_4 + 2C_6H_3OH(NH_2)COOH$$
Amino compound

All nitrogen compounds are then reduced to ammonium sulfate by sulfuric acid. Potassium sulfate crystals are added to increase the boiling temperature of the acid and insure a complete breakdown of the organic matter.

After the digestion is complete, the solution is made alkaline and the ammonia distilled into boric acid.

$$(NH_4)_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O + 2NH_3$$
$$3NH_3 + H_3BO_3 = (NH_4)_3BO_3$$

The amount of ammonia, all of which has reacted with the boric acid, is determined by titration with 0.5N hydrochloric acid.

$$(NH_4)_3BO_3 + 3HCl = H_3BO_3 + 3NH_4Cl$$

The amount of nitrogen equivalent to 1 ml. of the 0.5N acid is determined as follows:

Equivalent weight of N = 14

1 liter of 0.5N acid is equivalent to $14 \times 0.5 = 7.0$ gm. N or 1 ml. is equivalent to 7.0 mg. N

Potash.

Procedure, page 77.

The potash determination in sludge is usually omitted because of its low value. The sludge sample is decomposed with sulfuric

acid and ashed in the muffle furnace. Sulfuric acid changes all potassium salts to sulfates, which are not so readily decomposed by high temperatures as are other salts of potassium. The resulting ash is dissolved in water and hydrochloric acid. The potassium is precipitated with chloroplatinic acid.

$$K_2SO_4 + H_2PtCl_6 = K_2PtCl_6 + H_2SO_4$$

The precipitate is washed free of all excess chloroplatinic acid by means of 80 per cent alcohol. It is then dissolved in water, acidified with hydrochloric acid and the platinum precipitated as metallic platinum by magnesium.

$$K_2PtCl_6 + 2Mg = Pt + 2KCl + 2MgCl_2$$

The platinum is weighed and its weight converted to its equivalent as K_2O.

Calculations.

Molecular weight of K_2O = 94.20
Atomic weight of Pt = 195.23
(1) Weight of Pt × 94.20/195.23 = weight K_2O in sample
(2) Weight K_2O in sample × 100/Weight of sample = per cent K_2O
Combining 1 and 2

$$\frac{\text{Weight Pt} \times 94.20 \times 100}{\text{Weight of sample} \times 195.23} = \text{per cent } K_2O$$

Sewage Gas Analysis.

Procedure, pages 78 to 80.

The analysis of gas produced by the digestion of sewage sludge should include the determination of carbon dioxide and methane and a calculation of the B.t.u. value. The determination of hydrogen may be included, but the other gases, such as oxygen, carbon monoxide and nitrogen, are present in such small amounts that their determination as a routine procedure is of little value.

There are many types of apparatus on the market for the analysis of gas, but no type which is designed especially for sewage gas. Most of the apparatus is designed for a complete analysis and can be used by omitting the parts not required. The apparatus should contain a water-jacket measuring pipette, an explosion or burning pipette (preferably the latter) and a pipette for carbon dioxide.

In the CO_2 measurement, its absorption by the potassium hydroxide is represented by the following equation:

$$CO_2 + 2KOH = K_2CO_3 + H_2O$$

In the hydrogen and methane determination, the composition and volume of these gases are affected by burning according to the following equations:

$$2H_2 + O_2 = 2H_2O$$
$$CH_4 + 2O_2 = CO_2 + 2H_2O$$

Then the CO_2 produced is absorbed by the potassium hydroxide solution as was done in the carbon dioxide determination, resulting in a further reduction in volume equal to the volume of methane.

Problems.

1. A humus determination on 1.150 gm. of a dried sewage sludge showed a loss on ignition at 900°C. of 0.4150 gm. A calcium test on residue after ignition showed the presence of 0.0650 gm. of CaO. Compute the per cent humus and the per cent $CaCO_3$ in the sludge. *Ans.* 32.6 and 10.4 per cent.

2. The dried weight of a sewage-sludge sample used in a phosphoric acid determination was 2.1000 gm. The ammonium phosphomolybdate was dissolved in 10 ml. of 0.3238N sodium hydroxide and the excess sodium hydroxide required 4.2 ml. of 0.3238N hydrochloric acid. Compute per cent P_2O_5. *Ans.* 1.07 per cent.

3. A dried sewage-sludge sample for total nitrogen determination weighed 1.2000 gm. In the final titration it took 6.5 ml. of 0.5N hydrochloric acid. *a.* What was the per cent N in the sample? Express answer also in terms of NH_3. *b.* If the sample was dried from sludge, from a sludge bed containing 60 per cent moisture, compute the N and NH_3 values in the moist sludge. *Ans. a.* 3.79 and 4.59 per cent.
b. 1.52 and 1.84 per cent.

4. Prove the equations given in the hydrogen and methane determinations on page 80.

INDUSTRIAL WASTE ANALYSIS

Cyanides.

Procedure, page 82.

The cyanides have the property of oxidizing phenolphthalin to phenolphthalein which in alkaline solution is pink. The test is not specific for cyanides.

Acids and Iron in Trade Wastes.

Procedure, page 83.

If the waste is concentrated, which is often the case, a measured volume of the waste is first diluted with a definite volume of water (as 10 ml. of waste with 90 ml. of water). This allows a more accurate measurement of the volume of sample used since the accuracy of volumetric measurements increases with the increase in volume.

A measured amount of standard (0.2N) sodium hydroxide, in excess of that necessary to neutralize the acid and precipitate the iron, is added to the diluted sample.

$$FeSO_4 + 2NaOH = Fe(OH)_2 + Na_2SO_4$$
$$H_2SO_4 + 2NaOH = Na_2SO_4 + H_2O$$

The iron hydrate is removed by filtration and the excess sodium hydroxide determined by titration with standard (0.1N) sulfuric acid. The amount of combined iron and acid present is calculated from the quantity of sodium hydroxide used and the results expressed in terms of H_2SO_4.

Calculations.

(1) Ml. 0.2N NaOH added − (0.5 × ml. 0.1N H_2SO_4) = ml. 0.2N NaOH used
(2) 1 ml. 0.2N NaOH is equivalent to 0.00981 gm. H_2SO_4
(3) Ml. 0.2N NaOH × 0.00981 = gm. of acid and iron in sample expressed as H_2SO_4
(4) Gm. H_2SO_4 in sample × 1,000/Ml. of sample used = gm. H_2SO_4 per liter
(5) Ml. of sample used = ml. portion of diluted sample used/10

Combining above steps:

$$\frac{\text{Ml. 0.2N NaOH} - (0.5 \times \text{ml. 0.1N } H_2SO_4) \times 0.00981 \times 1{,}000 \times 10}{\text{Ml. portion of diluted sample used}} =$$

gm. H_2SO_4 per liter

A determination of the quantity of iron in the sample is then made by oxidizing the ferrous to ferric iron and precipitating ferric hydroxide with ammonium hydroxide. The precipitate is ignited and weighed as ferric oxide (Fe_2O_3). This weight is converted to its equivalent as ferrous sulfate ($FeSO_4$).

Calculations.

(1) Molecular weight of Fe_2O_3 = 159.68
Molecular weight of $FeSO_4$ = 151.90
(2) Each Fe_2O_3 is equivalent to $2FeSO_4$
(3) Weight Fe_2O_3 (gm.) $\times \frac{2 \times 151.90}{159.68}$ = gm. $FeSO_4$ in sample
(4) Gm. $FeSO_4$ in sample $\times$ 1,000/Ml. of sample used = gm. $FeSO_4$ per liter
(5) Ml. portion diluted sample used/10 = ml. sample used
Combining above steps:

$$\frac{\text{Weight } Fe_2O_3 \times 303.80 \times 1{,}000 \times 10}{159.68 \times \text{ml. portion diluted sample used}} = \text{gm. } FeSO_4 \text{ per liter}$$

The weight of H_2SO_4 in the sample is equal to the difference between the weight of the combined acid and iron expressed as H_2SO_4 and the weight of $FeSO_4$ expressed as H_2SO_4. $FeSO_4$ is converted to terms of H_2SO_4 as follows:
(1) Molecular weight of $FeSO_4$ = 151.90
Molecular weight of H_2SO_4 = 98.08
(2) Gm. $FeSO_4 \times 98.08/151.90$ = gm. $FeSO_4$ expressed as H_2SO_4

Iron, Volumetric Method.

Procedure, page 84.

The iron is first changed entirely to ferric sulfate [$Fe_2(SO_4)_3$] by the addition of sulfuric acid. All other acid radicals, such as chlorides, are volatilized by heating until the white fumes of sulfuric acid are obtained. These acid radicals may interfere with subsequent reactions.

The iron is then completely reduced to the ferrous state with zinc in acid solution.

$$H_2SO_4 + Zn = H_2 + ZnSO_4$$
$$Fe_2(SO_4)_3 + H_2 = 2FeSO_4 + H_2SO_4$$

The quantity of iron present is determined by titration with standard potassium permanganate.

$$2KMnO_4 + 10FeSO_4 + 8H_2SO_4 = 2MnSO_4 + K_2SO_4 + 5Fe_2(SO_4)_3 + 8H_2O$$

The iron is calculated in terms of Fe as follows:

(1) The change in valence of Fe in the above equation is from 2 to 3. Therefore, the equivalent weight is equal to the atomic weight, or = 55.84
(2) 1 ml. of 0.1N $KMnO_4$ is equivalent to 0.005584 gm. Fe
(3) $\frac{\text{Ml. of 0.1N } KMnO_4 \times 0.005584 \times 1{,}000}{\text{Ml. of sample used}}$ = gm. Fe per liter

Alkalies.

Procedure, page 85.

The reactions involved in this determination are discussed under Alkalinity, page 140. The results are expressed in terms of the weight of the hydroxide (OH), carbonate (CO_3) and bicarbonate (HCO_3) radicals, rather than as $CaCO_3$ as is done in the alkalinity determination. The factors used for these calculations are developed as follows:

(1) Equivalent weight of OH = 17
1 ml. of 0.1N H_2SO_4 = 0.0017 gm. OH
(2) Equivalent weight of CO_3 = 30
1 ml. of 0.1N H_2SO_4 = 0.0030 gm. CO_3
(3) Equivalent weight of HCO_3 = 61
1 ml. of 0.1N H_2SO_4 = 0.0061 gm. HCO_3

Phenols, Gibb's Method.

Procedure, page 86.

The water or waste is first acidified with phosphoric acid and the phenol distilled to eliminate interfering substances. An accurate adjustment of pH is necessary before the determination is made. This is done by the use of phosphate buffer solutions. A pH of 9.6 is desired.

Phenols, Bromine Method.

Procedure, page 88.

The sample is made alkaline with sodium hydroxide and the ammonia (NH_3) removed by boiling.

$$(NH_4)_2SO_4 + 2NaOH = 2NH_3 + 2H_2O + Na_2SO_4$$

Hydrogen sulfide as well as other sulfides are removed by precipitation with lead carbonate.

$$H_2S + PbCO_3 = H_2O + CO_2 + PbS$$

The solution is neutralized with sodium bicarbonate and the phenol (C_6H_5OH) distilled to remove interfering substances. An excess of 0.1N bromine solution is added and the solution acidified.

$$C_6H_5OH + 3Br_2 = C_6H_2Br_3OH + 3HBr$$

Potassium iodide (KI) is added and the excess bromine is determined by titrating the liberated iodine with 0.1N sodium thiosulfate ($Na_2S_2O_3$).

$$2KI + Br_2 = 2KBr + I_2$$
$$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI$$

Calculations.

(1) Molecular weight of phenol = 94.0
(2) According to the above reaction one C_6H_5OH molecule reacts with six bromine atoms
(3) Equivalent weight of phenol = 15.67
(4) 1 ml. of 0.1N bromine = 0.001567 gm. or 1.567 mg. of phenol
(5) Ml. 0.1N bromine − ml. of 0.1N thiosulfate used = ml. of 0.1N bromine used by the phenol
(6) $\frac{\text{Ml. of 0.1N bromine} \times 1.567 \times 1{,}000}{\text{Ml. of sample used}} = \text{p.p.m. phenol}$

ANALYSIS OF CHEMICALS

Lime, Percentage of Oxide or Hydroxide.

Procedure, page 44.

The reactions involved in this determination are discussed under Alkalinity, page 140. The factors used in the calculations are developed as follows:

(1) 0.5 gm. of lime sample was dissolved in 500 ml. of water and a 50-ml. portion of this solution used for the titration. Then weight of lime sample titrated = 0.05 gm.
(2) Equivalent weight of $Ca(OH)_2$ = 37.0
(3) 1 ml. of 0.1N H_2SO_4 = 0.0037 gm. $Ca(OH)_2$
(4) $\frac{\text{Ml. of 0.1N } H_2SO_4 \times 0.0037 \times 100}{0.05} = \text{per cent } Ca(OH)_2$
(5) Equivalent weight CaO = 28.0
(6) 1 ml. of 0.1N H_2SO_4 = 0.0028 gm. CaO
(7) $\frac{\text{Ml. of 0.1N } H_2SO_4 \times 0.0028 \times 100}{0.05} = \text{per cent CaO}$

Lime, Percentage of Calcium.

Procedure, page 45.

The reactions involved in this determination are discussed under Calcium, page 160. The factors used in the calculations are developed as follows:

(1) Equivalent weight of CaO = 28.0
(2) Equivalent weight of $Ca(OH)_2$ = 37.0
(3) 37.0/28.0 = 1.32
(4) Gm. CaO × 1.32 = gm. $Ca(OH)_2$

Alum, Per Cent Aluminum Oxide.

Procedure, page 46.

The reactions involved in this determination are discussed under Iron and Aluminum Oxides, page 159. Since the Al_2O_3 is weighed direct, no factor is necessary in making the calculations.

Chlorine in Hypochlorites.

Procedure, page 47.

Hypochlorites of calcium and sodium are usually represented by the formulas $CaCl_2O$ and NaClO, respectively. These compounds are readily decomposed by acids, even carbonic acid, liberating chlorine.

Because of the ease with which chlorine is lost if the sample is exposed to the air during analysis, the sample is poured into water in a beaker. The addition of acetic acid liberates the chlorine.

$$CaCl_2O + 2CH_3COOH = Ca(CH_3COO)_2 + Cl_2 + H_2O$$

The free chlorine reacts with the potassium iodide, liberating an equivalent amount of iodine.

$$2KI + Cl_2 = 2KCl + I_2$$

The iodine is titrated with 0.025N sodium thiosulfate and the amount of chlorine in the sample calculated as follows:

(1) Equivalent weight of chlorine is 35.456
(2) 1 ml. of 0.025N thiosulfate = 0.8864 mg. Cl
(3) 25 ml. portion of 200 ml. solution used
(4) $\frac{\text{Ml. 0.025N thiosulfate} \times 0.8864}{1{,}000}$ = gm. chlorine in 25-ml. portion
(5) $\frac{\text{Gm. chlorine in 25-ml. portion} \times 200 \times 100}{\text{Gm. sample used} \times 25}$ = per cent chlorine in sample

Soda Ash, Per Cent Sodium Carbonate.

Procedure, page 46.

The reaction involved in the titration is represented by the following equation:

$$\underset{98.08}{H_2SO_4} + \underset{106.10}{Na_2CO_3} = \underset{141.16}{Na_2SO_4} + \underset{62.02}{H_2CO_3}$$

The equivalent weight of H_2SO_4 = 49.04

The equivalent weight of Na_2CO_3 = 53.05

0.1N H_2SO_4 contains 0.004904 gm. H_2SO_4 per milliliter

$$\text{Gm. } Na_2CO_3 \text{ in sample} = 5 \times \frac{53.05}{49.04} \times 0.004904 \times \text{ml. of acid}$$

$$= 5 \times 0.005305 \times \text{ml. of acid}$$

$$\text{Per cent } Na_2CO_3 = \frac{5 \times 0.005305 \times \text{ml. of acid} \times 100}{0.53} = 5 \times \text{ml. of acid}$$

Problems.

1. A 0.35-gm. sample of dried hydrated lime was weighed for CaO test by method given on page 45. The gain in weight after ignition was found to be 0.24 gm. Compute the per cent calcium, as $Ca(OH)_2$ and as CaO, in the sample. *Ans.* 90.6 and 68.6 per cent.

2. An analysis of soda ash was made on a 0.53-gm. sample by the method given on page 46. It took 19.6 ml. of 0.1N sulfuric acid in the last step of the determination. Compute the per cent Na_2CO_3.

Ans. 98.5 per cent.

3. A 0.5000-gm. sample of alum was tested by method given on page 46. The gain in weight in the last step of the test was 0.087 gm. Compute per cent Al_2O_3 and $Al_2(SO_4)_3$. *Ans.* 17.4 and 58.3 per cent.

DIVISION II

SPECIAL TOPICS

SAMPLING

Sampling of a water, sewage or trade waste must be accomplished with proper precautions to secure a representative sample. Too often the error in sampling is inconsistent with the accuracy of the determinations made in the laboratory. It is seldom sufficient to rely on the results of a single ("grab") sample. It is more often necessary to use a composite sample, made up of a number of individual samples, or to use the results of analyses of a number of individual or composite samples. Good judgment must be exercised in any case in selecting the sampling method to be used, the selection of the method often being influenced by the laboratory facilities available. It is evident that the results of a laboratory analysis, however accurate that analysis may be, cannot represent an accuracy for the material sampled of a greater degree than the accuracy with which the sample was taken.

In the collection of representative samples, the following points must be taken into consideration:

1. The character of the laboratory examinations to be made.
2. The use to be made of the results of the analysis.
3. The character of the material sampled and the variation in character over the period of sampling.
4. The variation in the rate of flow over the period of sampling.

Samples for the ordinary chemical analysis of water and sewage should be collected in clean glass-stoppered bottles. The size of the sample collected depends upon the analysis to be made.

Well-water Samples.

Well waters are usually quite constant in character and composite sampling is not necessary in order to obtain representative

samples. Before the sample is taken the well should be pumped for some time so that the sample will represent the ground water from which the well is fed. The container must be rinsed with the water and then completely filled. If an analysis is to be made for dissolved gases, carbon dioxide and oxygen, this analysis should be made immediately.

Ponds and Lakes.

Ponds and lakes are often subject to variable conditions resulting from natural causes, such as seasonal turnovers, rains and winds. This variation is usually not so rapid nor over such a wide range as is encountered in a flowing body of water. Single samples are often sufficiently representative in these cases, but they should be taken often enough to take into account those variations which are likely to occur.

Flowing Streams.

Flowing streams are subject to considerable variation and in most cases require composite sampling or the averaging of the results of the analyses of a large number of samples. Samples from a stream should be taken at a point which most nearly represents the conditions in the stream. This point should be within the flowing channel and at about mid-depth. Wide, deep streams may require the collection of samples in a number of verticals across the stream and at several depths in the verticals in order to obtain a representative sample. Composite samples made up of hourly individual samples taken according to flow are usually considered as representative of the stream condition.

Water Treatment Plants.

In water treatment plant control, individual samples or composites over short periods of time must be taken at frequent intervals and at such points in the treatment process as to determine the quantity of chemicals required and the accomplishments obtained. The exact schedule will vary with the particular conditions at each plant. For instance, a raw water supply from wells may require but one sample a day, while a supply from a stream may require hourly sampling. Many laboratories

have provisions for taking of constant samples at all points in the process, the particular water being piped to the laboratory.

Sewage and Sewage Treatment Plants.

In sampling influents and effluents from sewage treatment plants or sewage from a sewerage system it is particularly important to take composite samples. The character of sewage is too variable in short intervals of time to place any reliance upon individual samples. Composite samples should be made up of individual samples which vary in size according to the relative flows at the time they are taken.

It is desirable to extend the taking of the composite over a 24-hr. period or, in smaller plants, at least over several hours. A better study of plant accomplishments may be made by dividing the 24 hr. into two or more groups and obtaining a composite for each group.

Sewage samples may be readily collected by means of dippers or cans several inches in diameter. The dipper should be immersed well into the sewage and the sample taken at about mid-depth. The dippers may be made of various sizes to conform to the flows obtained. Each individual sample should be deposited in a larger receptacle of sufficient size to hold the composite. The composite should be kept at a low temperature in order to inhibit bacterial action and prevent as much change in character as is possible.

Preservation of Sewage Samples.

Samples of sewage will change considerably upon standing. Methods of preservation decrease the rate of change to some extent and should be used wherever possible. Since the time factor is all-important, the samples should be analyzed soon after collection. Composite samples, on which the B.O.D. and suspended-solids tests are to be made, should be kept at a low temperature (as near 4° C. as possible).

A preservative cannot be used if the B.O.D. test is to be made unless this preservative is later removed and the sample or B.O.D. dilution reseeded. Samples for solids tests may be partially preserved by adding about 5 ml. of chloroform per liter. The addition of sulfuric acid is not favored because of its effect on

precipitated carbonates and other acid-soluble solids. Gaseous chlorine or strong chlorine water may be used as a satisfactory preservative, except when bacterial and B.O.D. tests are to be made.

Trade Wastes.

The methods for sampling trade wastes must be suited to the particular waste. If the rate of flow and character of the liquid are both variable, the procedure should be similar to that used for sewage. In some cases the flow and character are constant, requiring only an individual sample. Often there are times of periodic or occasional discharges of strong wastes. An inspection of the trade processes should be made before any samples are taken in an endeavor to ascertain the methods of waste discharge.

Sampling for Dissolved Oxygen.

It is necessary to make dissolved-oxygen determinations on samples at the time of collection. This makes necessary the use of individual samples for this test. Samples should be taken with extreme care so as to avoid contact of the sample with air. In order to facilitate the taking of dissolved-oxygen samples, a sampling can similar to that shown in Fig. 2 may be used. This can should be of such a size that the displacement in the can is at least four times that of the sample bottle or bottles. The use of this can prevents contact of the sample with air. The can shown with provision for two bottles is convenient for the taking of stream-survey samples because it is often desired to use one bottle for an immediate dissolved-oxygen test and the other bottle for a biochemical oxygen-demand test.

Sampling of Sewage Sludge.

Samples of wet sludge being drawn from a tank should be composited from individual samples. A regular schedule should be adopted for individual collections at various times during the drawing operation. Five-minute intervals are often used. The individual samples should be placed in a large receptacle and well mixed, a smaller sample being taken for the laboratory.

If sludge from a drying bed is to be sampled, a composite should be made up by taking individual samples from various

parts of the bed. The bed should be divided into a number of sections and individual samples taken from the full depth of the

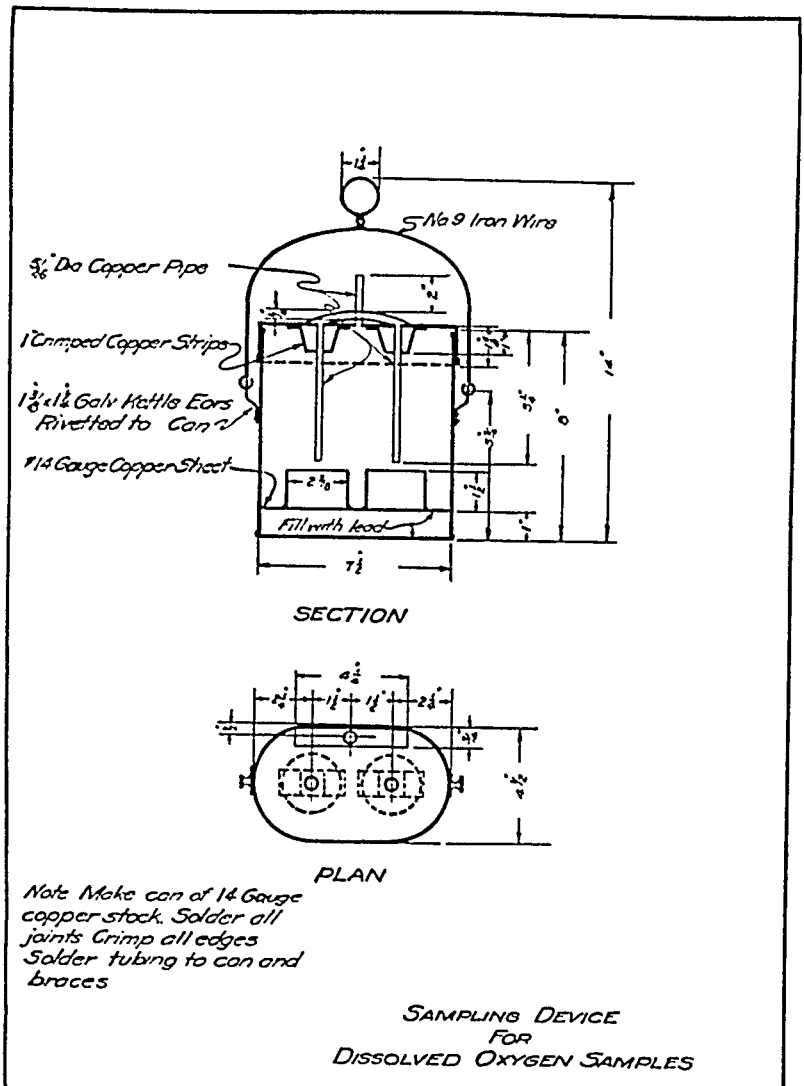


FIG. 2.

material in each section. This may be done by forcing a 1-in. thin-walled metal tube into the sludge the full depth, withdraw-

ing the tube and removing the adhering sand. A plunger placed in the tube will facilitate the removal of the sample. All of the individual samples should be well mixed. The final sample for analysis should be obtained by quartering the composite. Quartering consists of dividing the pile on the paper into quarters, discarding opposite quarters, again mixing, quartering and discarding until the required size is obtained.

Sampling of Dry Material.

Samples of lime, soda ash, alum, dried sewage sludge, filter sand, etc., must be taken in such a manner as to be representative of the entire mass from which they are taken. Individual samples, taken from various locations in the material, should be composited. This composite should be dried and ground, if necessary, to the degree of fineness required for the analysis. The sample should then be thoroughly mixed. This may be done conveniently by placing it on a large sheet of paper and by raising alternate corners, rolling the material diagonally across the paper. If the sample is too large for analysis, it must be divided by quartering.

STRENGTH OF SEWAGE

Sewage is defined by the A.P.H.A. and A.S.C.E. as: A combination of wastes (*a*) from residences, business buildings and institutions and (*b*) from industrial establishments, with (*c*) such ground, surface and storm water as may be admitted or find its way into the sewers. Total solids are about 0.1 of 1 per cent by weight. Generally less than 400 p.p.m. of the solids is volatile, and it is the volatile solids which usually cause the

STRENGTH OF SEWAGES
(Values in Parts Per Million)

Item	Weak	Medium	Strong
*Total solids	Less than 650	600–900	Greater than 850
Total volatile solids	Less than 350	300–500	Greater than 450
Suspended solids	Less than 175	150–250	Greater than 225
Suspended volatile solids	Less than 115	100–170	Greater than 150
5-day B.O.D	Less than 150	125–225	Greater than 200

* Includes 50 p.p.m. solids in water supply.

nuisances from sewage. Sewage is assumed to weigh the same as water (8.34 lb. per gallon).

The strength of sewages is indicated by the preceding tabulation. Solids in water supplies are usually nearly all mineral (nonvolatile) and may range from 50 p.p.m. or less to several hundred p.p.m. and will increase the total solids in the sewage correspondingly.

LABORATORY EQUIPMENT

The Analytical Balance.

The analytical balance is the most important piece of equipment in the laboratory and should be treated accordingly. The following directions must be carefully followed if correct weights are to be obtained and the sensitivity of the balance used to its fullest extent.

Before using, clean the balance floor and pans with a camel's-hair brush. While not in use, raise the balance beam and pans from their knife-edge supports by means of the milled knob outside of the balance case. Also raise the pan rests against the pans by means of the knob for that purpose. After cleaning, lower the balance beam and pans slowly to their knife-edge supports. Release the pan rests and notice if the pointer swings equal distances on each side of the zero of the scale. If it does not swing equal distances (within less than one division), it may be adjusted by the adjusting screws, or the mean position of the swings may be determined and used as the zero point.

With the balance beam and pans lifted from their knife-edge supports and the pan rests against the pans, place the object to be weighed in the left-hand pan and release the pan rests. If the pans swing, at any time, they may be stopped by lowering and raising the pan rests. Place a weight in the right-hand pan which you judge is just heavier than the object being weighed. Lower the beam support slowly. If the weight is too light (pointer moves to right), raise the beam support and replace the weight with the next heavier one and repeat this operation until the first weight is found that is too heavy. Replace this weight by the next lighter weight and this will be the first weight to be retained on the balance pan. Continue adding the next consecutive lighter weight and testing by releasing the beam

support after each weight addition. When the weights smaller than 1 gram are used, release the beam support and use the pan rests. Continue adding weights and testing until within the range covered by the rider. Close the balance-case door and complete the weighing with the rider.

Do not touch objects to be weighed, the balance or weights with the hands. Handle the weights with the weight forceps and carefully return them to their proper places in the box. Place the fractional weights right side up with the bent corner at the right. Always count the weights twice and notice if they check with those missing from the box. Before leaving the balance have all weights returned to the box, the rider removed from the beam, the beam and pans lifted off their knife-edge supports, the pan rests raised and the pans and balance floor clean.

Glassware, Its Use and Care.

Volumetric glassware, in particular, should be kept clean and free-draining. If the glassware does not drain free and dry (no drops of water adhering to the glass), it should be cleaned with cleaning solution and rinsed several times with distilled water.

The standard procedure for delivering the calibrated volume from a pipette is as follows: (1) After filling to the mark allow the pipette to drain normally (do not blow into it). (2) Hold it above the liquid for 15 sec. to allow it to drain. (3) Touch the tip of the pipette to the surface of the liquid, making no further attempt to remove the solution which remains in the tip.

All glassware should be cleaned with cleaning solution or powders, rinsed thoroughly with distilled water and allowed to drain dry. Hydrochloric (muriatic acid) is often more effective in cleaning than cleaning solution. This is particularly the case where the deposits (iron and carbonates) from hard water occur. Cleaning should be done as soon as possible after use so that the apparatus is always dry and ready for use.

Care and Use of Platinum.

Platinum dishes are very desirable for solids and loss on ignition determinations. Platinum fuses at 1770°C. but does

not soften to any extent at the temperatures used for ignition. It resists the action of all single acids if pure. It is dissolved by chlorine solutions and, therefore, by aqua regia. Hydrochloric acid often contains traces of free chlorine which will attack the platinum.

Platinum alloys with most metals, especially those in compounds which are easily reduced. When heated for a long time in contact with carbon it becomes brittle, forming carbide of platinum. This is noticed when a dish is heated in the reducing flame of a burner. For this reason the tip of the inner cone of a burner flame should always be below (not against) the bottom of a platinum dish.

The following precautions should be observed when using platinum:

1. Do not heat with compounds of lead, tin, zinc, alkali hydroxides and other compounds of metals which are easily reduced.
2. When igniting with a burner keep the inner cone of the flame below the bottom of the dish. (Avoid use of the yellow flame.)
3. Handle carefully and avoid bending. Use platinum-tipped tongs.
4. For cleaning use a solvent suitable for the material to be removed. Cleaning solution should be used for organic matter, hydrochloric acid for insoluble carbonates or metallic oxides, fusion with sodium carbonate for silica and fusion with potassium bisulfate for such metallic compounds as will not be removed by acids.
5. Dull surfaces should be polished lightly with wet emery slime or round sand. Do not scrape with files or glass rods.

FORMATION AND TREATMENT OF PRECIPITATES

Gravimetric determinations in most cases depend upon the precipitation of a very slightly soluble compound, filtering the same, freeing the precipitate of soluble compounds and drying or igniting and weighing. Certain precautions must be exercised in these procedures. One of the first is the formation of a precipitate of sufficiently large particles so as to be retained on the filter paper. Heating, applying the precipitating reagent slowly and stirring constantly are all factors in the production of large

particles. The application of the precipitating agent slowly produces a small number of particles upon which the remaining precipitate grows. Heating and stirring tend to redissolve the smaller particles and reprecipitate them on the larger ones resulting in the formation of larger particles.

If the precipitate is to be ignited and weighed, it must be filtered onto quantitative filter paper, that is, paper having a low ash content. The weight of ash in a 9 cm. quantitative filter paper should not be greater than 0.00005 to 0.0001 gm.

Before a precipitate is weighed it must be washed free from the soluble impurities. The first washings may be made in the precipitation vessel by decantation. To accomplish this, the major portion of the precipitate is allowed to settle and the supernatant solution decanted through the filter paper. A small portion of wash water is added, mixed thoroughly with the precipitate, and this is decanted through the paper. After several washings of this kind, the precipitate is entirely transferred to the paper and again washed with several small portions of wash water. Many small portions of wash water are much more effective in washing than a few larger portions for the same total volume used.

After the precipitate is filtered and washed, the paper must be folded, placed in the crucible and dried and charred before ignition. Usually the ignition, in the event no muffle furnace is available, should be carried out in the oxidizing flame of a burner of the Meeker type. The oxidizing flame is that portion outside of the inner cone of the flame. The inner cone of a gas flame is a reducing flame. Before ignition the paper must be dried and completely charred in a low flame. If the burner is used the flame is increased and the crucible inclined on its side to facilitate the ignition of the carbon of the paper. The heating should not be rapid enough to cause the paper to burst into flame. If this happens, immediately smother the flame by covering the crucible. From this point on the ignition may be carried out in a muffle furnace or over a burner.

RECORDS

All data, including weighings, calculations and details of computations, should be recorded in some form of bound note-

book. This permits a ready reference to all previous records and makes all laboratory data available at any time for checking of calculations and results.

Considerable time can be saved in routine analysis if these notebooks are made up locally and patterned after a form to comply with the specific needs of the laboratory. All data should be entered on the right-hand pages of the book, reserving the left-hand pages for calculations. Most plants develop record forms in which all of the laboratory data as well as other plant data are compiled. These data may be in the form of a weekly, monthly or yearly record. These forms, also, must be made to fill the specific needs.

DIVISION III

TABLES

TABLE 1.—LIST OF APPARATUS

Name	Number of pieces					
	A*	B	C	D	E	F
Analytical balance	..	1	1	..	1	1
Balance weights	..	1	1	..	1	1
Beakers, 2-liter	..	2	4	..	..	2
Beakers, 1-liter	..	4	6	..	2	2
Beakers, 600-ml	..	4	6	..	..	2
Beakers, 400-ml	..	2	4	..	6	
Beakers, 250-ml	..	6	12	..	6	6
Beakers, 50-ml	..	3	4	..	..	2
Beaker covers, 75-mm	..	6	12	..	..	6
Bottles, glass-stoppered, 32-oz	6	24	36	..	6	24
Bottles, glass-stoppered, 16-oz	4	8	12	..	3	12
Bottles, glass-stoppered, 8-oz	..	8	12	12	24	72
Burettes, 50-ml	..	8	12	..	2	4
Burette support	..	1	2	..	1	2
Burners, Meeker	..	2	2	..	2	2
Burners, Bunsen	..	2	6	..	2	6
Clamps, burette	..	6	6	..	2	4
Clamps, condenser	..	..	..	..	..	2
Clamps, holders	..	..	..	..	..	2
Clamps, pinch	..	12	12	..	4	6
Condenser	..	..	..	..	..	1
Corks, bag	2	4	6	..	2	3
Cork borers	.	1	1	..	1	1
Crucibles, 46-mm	.	4	12	..	..	6
Crucibles, Gooch, 25-ml	..	..	..	..	4	18
Crucible, Gooch, holders	..	..	..	..	2	4
Crucible tongs	..	4	6	..	2	4
Crucible tongs, 18-in. handle	..	..	1	..	1	1
Desiccators, 250-mm	..	1	3	..	1	2
Desiccator plates, 230-mm	..	1	3	..	1	2
Dropping bottles, 60-ml	..	3	6	..	4	6
Drying oven	..	..	1	..	1	1
Erlenmeyer flasks, 500-ml	..	6	8			
Erlenmeyer flasks, 250-ml	..	12	24	..	4	12
Evaporating dishes, 90-mm	..	4	6	..	2	2
Evaporating dishes, 75-mm	..	6	12	..	4	12
Evaporating dish, platinum	..	2	2			
Files	..	2	2	..	1	2
Filter paper, 12-cm., packages	..	1	2			
Filter paper, 9-cm., packages	..	6	12	..	..	6
Filter paper, quantitative, 9-cm	..	..	2	.	..	1
Funnels, 90-mm	..	2	2			
Funnels, 65-mm	..	6	12	..	..	6
Funnels, Büchner, 80-mm	..	..	..	..	..	1
Funnel support	..	1	1	..	1	1
Gas analysis apparatus	..	..	..	..	..	1
Glass rods, 6-mm., (lb.)	..	1	2	..	1	1
Glass tubing, 7-mm., lb	..	5	10	..	1	3
Graduated cylinders, 1-liter	..	2	4	..	2	2
Graduated cylinders, 100-ml	..	2	6	..	2	2
Graduated cylinders, 25-ml	..	2	4	..	1	2
Graduated cylinders, 10-ml	..	2	4	..	1	2
Hydrogen-ion apparatus	..	1	1	1	1	1
Imhoff cones	..	..	..	3	3	4
Incubator 20°C	..	..	..	..	1	1
Iron supports	..	3	6	..	1	6
Jar brush	..	1	2	..	2	2
Kjeldahl flasks, 500-ml	..	..	..	..	..	4
Kjeldahl flasks, bulbs	..	..	..	..	..	4
Mortar and pestle	..	1	1			
Muffle furnace	..	..	1	..	1	1

* For significance of these letters see explanation below.

TABLE 1.—LIST OF APPARATUS.—(*Continued*)

Name	Number of pieces					
	A*	B	C	D	E	F
Nessler tubes, 100-ml	8	12	12	3	3	24
Nitrogen distillation apparatus	..	..	..	..	..	1
Pipettes, 100-ml	..	4	6	..	2	2
Pipettes, 50-ml	..	4	6	..	..	2
Pipettes, 25-ml	..	4	6	..	..	2
Pipettes, 10-ml	..	4	6	..	..	2
Pipettes, 5-ml	..	..	..	..	..	2
Pipettes, 1-ml	4	6	12	2	5	12
Pipettes, graduated 1/100, 1-ml	..	..	..	..	2	6
Pipettes, graduated 1/10, 10-ml	..	2	6	..	2	6
Pipettes, graduated 1/10, 5-ml	..	..	6			
Platinum tipped tongs	..	1	1			
Rings, iron, 4-in	..	2	4	..	2	6
Rings, iron, 3-in	..	2	6			
Rubber tubing, ¼-in., (ft.)	..	30	50	..	24	48
Rubber stoppers, assorted, (lb.)	..	5	10	..	1	2
Sampling can (D.O.)	..	..	..	..	1	1
Scale weights	..	1	2	..	1	1
Soxhlett flask, 150-ml	..	..	..	..	2	2
Spatulas, 3 in	..	..	..	..	2	3
Suction flasks, 500-ml	..	..	..	..	2	4
Test tubes, assorted	..	24	36	..	36	72
Test-tube brush	..	2	3	..	3	3
Test-tube support	..	1	1	..	1	1
Thermometers, −10 to 110°C	..	2	4	..	2	2
Thermometers, 0 to 250°C	..	..	..	..	..	2
Triangles, 2-in	..	6	6	..	2	4
Tripods	..	2	2	..	1	4
Trip scales	..	1	2	..	1	1
Turbidimeter, Jackson	..	1	1			
Volumetric flasks, 1-liter	..	4	6	..	2	3
Volumetric flasks, 500-ml	..	..	2	..	..	2
Volumetric flasks, 250-ml	..	4	6	..	..	4
Volumetric flasks, 200-ml	..	6	12			
Volumetric flasks, 100-ml	..	2	4	..	2	2
Wash bottles, 1-liter	2	6	8	..	2	4
Water bath, 6 openings	..	1	1	..	1	1
Water suction pumps	..	..	..	..	2	4
Wire gauze, 4-in	..	6	6	..	..	4

* For significance of these letters see explanation below.

EXPLANATION OF TABLES NO. 1 and 2

Apparatus and chemicals have been listed in groups according to the type of treatment plants and determinations to be made. These are basic lists to be used in outfitting the various laboratories and may require additions or deductions to suit the special situations. The classification has been made as follows:

A. For water plants with chlorine treatment only.
B. For water plants having coagulation, filtration and chlorination.
C. For water plants having softening, coagulation, filtration and chlorination.
D. For sewage treatment plants in which settleable solids, relative stability, hydrogen-ion (pH) and chlorine determinations are made.
E. For sewage treatment plants where solid tests, oxygen determinations, pH and chlorine tests are made.
F. For sewage treatment plants in which all the usual control tests in this manual are made.

TABLE 2.—LIST OF CHEMICALS

Name	Quantity, pounds					
	A	B	C	D	E	F
Acetic acid, glacial						
Alizarine Red S. (gm.)	..	10	10			
Alcohol, grain (qt.)	..	1	1			
Aluminum sulfate crystals	..	1	1			
Ammonium carbonate	..	½	½			
Ammonium chloride	..	..	1	..	..	1
Ammonium hydroxide	..	4	8	..	..	8
Ammonium oxalate crystals	..	..	1	..	..	½
A-naphthylamine hydrochloride						
Asbestos fiber	..	..	..	..	¼	¼
Barium chloride	..	..	1			
Barium hydroxide	..	..	1			
Benzidine hydrochloride	..	¼c	¼			
Boric acid	..	..	..	..	..	1
Bromine	..	..	¼			
Calcium carbonate, C.P.	..	½	1			
Calcium hydroxide, C.P.	..	..	1			
Castile soap powder	..	1	1			
Chloroform	..	½	½		¼	¼
Cobaltous chloride	..	¼	¼			
Copper sulfate	..	..	..	..	..	1
2,6-dibromoquinonechloroimide						
Di-potassium phosphate						
Disodium phosphate	..	..	1			
Ferric ammonium sulfate	..	..	..	..	..	1
Ferrous ammonium sulfate	..	½	½			
Fuller's earth	..	1	1			
Glass wool	..	..	1			
Hydrochloric acid, C.P.	..	6	12	..	..	12
Hydroquinone	..	..	..	..	..	1
Hydroxylamine hydrochloride	..	¼c	¼			
Iodine	..	..	..	..	..	¼
Litmus paper (vials)	..	2	4	..	..	4
Manganous sulfate crystals	..	..	1	..	5	5
Magnesium ribbon	..	..	..	..	..	¼
Mercuric chloride	..	..	..	..	..	1
Methylene blue (gm.)	..	..	..	10	10	10
Methyl orange (gm.)	..	25	50	..	..	25
Molybdic acid	..	..	..	..	..	1
Monopotassium phosphate						
Nitric acid, C.P.	..	..	..	..	..	7
o-tolidine (gms.)	25	25	25	25	25	25
Phenol						
Phenolphthalein (gm.)	..	25	50	..	..	25
Phenolphthalin						
Platinic chloride						
Phosphoric acid						

TABLE 2.—LIST OF CHEMICALS.—(*Continued*)

Name	Quantity, pounds					
	A	B	C	D	E	F
Potassium bromate						
Potassium bromide						
Potassium biniodate						
Potassium chloride	..	..	..	..	..	1
Potassium chloroplatinate (gm.)	..	1	1			
Potassium chromate, C.P.	..	¼	¼	..	..	1
Potassium dichromate, C.P.						
Potassium dichromate, Tech.	..	5	10	..	5	5
Potassium ferricyanide						
Potassium hydroxide	..	..	..	..	..	2
Potassium iodide	..	1	2	..	1	1
Potassium nitrate	..	..	..	..	..	1
Potassium oxalate	..	..	..	..	..	1
Potassium permanganate	..	..	1	..	..	1
Potassium sulfate crystals	..	..	..	..	..	1
Potassium thiocyanate	..	½	½			
Salicylic acid	..	..	..	..	..	½
Silver nitrate, C.P.	..	¼	¼	..	..	½
Silver nitrite (oz.)	..	..	..	..	..	1
Silver sulfate						
Sodium bicarbonate	..	1	1	..	1	1
Sodium carbonate, anhydrous	..	½	1	..	..	1
Sodium chloride, C.P.	..	½	1	..	..	½
Sodium hydroxide, sticks	..	5	10	..	5	5
Sodium hypochlorite (oz.)	4	4	4			
Sodium oxalate, C.P.	..	..	1	..	..	1
Sodium thiosulfate crystals	..	½	1	..	1	1
Starch, corn	..	1	1			
Sulfanilic acid						
Sulfuric acid, C.P.	..	18	27	9	9	18
Sulfuric acid, Tech.	..	9	18	9	9	18
Sulfuric acid, fuming						
Thymolphthalein						

TABLE 3.—ATOMIC WEIGHTS

Element	Symbol	Atomic weight	Valence*	
			Positive	Negative
Aluminum	Al	26.97	3	
Barium	Ba	137.36	2	
Bromine	Br	79.916	...	1
Calcium	Ca	40.08	2	
Carbon	C	12.00	2, 4	4
Chlorine	Cl	35.457	...	1
Chromium	Cr	52.01	3, 6	
Copper	Cu	63.57	1, 2	
Hydrogen	H	1.008	1	
Iodine	I	126.920	...	1
Iron	Fe	55.84	2, 3	
Magnesium	Mg	24.32	2	
Manganese	Mn	54.93	2, 4, 7	
Molybdenum	Mo	96.00	6	
Nitrogen	N	14.008	3, 5	3
Oxygen	O	16.00	...	2
Phosphorus	P	31.02	5	
Platinum	Pt	195.23	4	
Potassium	K	39.096	1	
Silicon	Si	28.06	4	
Silver	Ag	107.88	1	
Sodium	Na	22.997	1	
Sulfur	S	32.06	4, 6	2

* Only those valences in common use in water and sewage chemistry are given.

TABLE 4.—MOLECULAR AND EQUIVALENT WEIGHTS

Compound	Formula	Molecular weight	Equivalent weight
Acetic acid	CH_3COOH	60.03	60.03
Alum, ammonium	$Al_2(SO_4)_3.(NH_4)_2SO_4.24H_2O$	906.64	151.11
Alum, crystals	$Al_2(SO_4)_3.18H_2O$	666.43	111.07
Alum, potassium	$Al_2(SO_4)_3.K_2SO_4.24H_2O$	948.76	159.79
Aluminum	A	26.97	8.99
Aluminum hydroxide	$Al(OH)_3$	77.99	26.00
Aluminum oxide	Al_2O_3	101.94	16.99
Aluminum sulfate	$Al_2(SO_4)_3$	342.14	57.02
Ammonia	NH_3	17.03	17.03
Ammonium chloride (sal ammoniac)	NH_4Cl	53.50	53.50
Ammonium hydroxide	NH_4OH	35.05	35.05
Ammonium nitrate	NH_4NO_3	80.05	80.05
Ammonium oxalate	$(NH_4)_2C_2O_4$	124.08	62.04
Ammonium oxalate, crystals	$(NH_4)_2C_2O_4.H_2O$	142.09	71.05
Ammonium sulphate	$(NH_4)_2SO_4$	132.14	66.07
Barium carbonate	$BaCO_3$	197.36	98.68
Barium chloride	$BaCl_2$	208.27	104.14
Barium chloride, crystals	$BaCl_2.2H_2O$	244.31	122.15
Barium sulfate (barium white)	$BaSO_4$	233.42	116.71
Boric acid	H_3BO_3	62.02	
Bromine	Br	79.92	79.92
Calcium bicarbonate	$Ca(HCO_3)_2$	162.10	81.05
Calcium carbonate (limestone)	$CaCO_3$	100.08	50.04
Calcium chloride	$CaCl_2$	110.99	55.50
Calcium hydroxide (slaked lime)	$Ca(OH)_2$	74.10	37.05
Calcium oxalate	CaC_2O_4	128.08	64.04
Calcium oxide (quicklime)	CaO	56.08	28.04
Calcium sulfate (gypsum)	$CaSO_4.2H_2O$	172.17	86.09
Calcium sulfate (Plaster of Paris)	$CaSO_4$	136.14	68.07
Carbon dioxide	CO_2	44.00	
Chlorine	Cl_2	70.914	35.457
Copper sulfate	$CuSO_4$	159.66	79.83
Copper sulfate (blue vitriol)	$CuSO_4.5H_2O$	249.71	124.86
Ferric chloride	$FeCl_3$	162.21	54.07
Ferric chloride, crystals	$FeCl_3.6H_2O$	270.30	90.10
Ferric hydroxide	$Fe(OH)_3$	106.86	35.62
Ferric oxide	Fe_2O_3	159.68	26.61
Ferric sulfate	$Fe_2(SO_4)_3$	399.86	66.64
Ferric sulfate, crystals	$Fe_2(SO_4)_3.9H_2O$	562.00	93.67
Ferric sulfocyanate	$Fe(CNS)_3$	230.04	76.68
Ferrous ammonium sulfate	$FeSO_4(NH_4)_2.6H_2O$	392.13	196.07
Ferrous carbonate	$FeCO_3$	115.84	57.92
Ferrous hydroxide	$Fe(OH)_2$	89.86	44.93
Ferrous sulfate	$FeSO_4$	151.90	75.95
Ferrous sulfate, crystals (copperas)	$FeSO_4.7H_2O$	278.01	139.00
Hydrochloric acid (muriactic acid)	HCl	36.47	36.47
Hydrogen	H_2	2.016	1.008
Hydrogen sulfide	H_2S	34.08	17.04
Iodine	I	126.92	126.92
Magnesium ammonium phosphate	$MgNH_4PO_4$	137.38	
Magnesium bicarbonate	$Mg(HCO_3)_2$	146.34	73.17
Magnesium carbonate (magnesite)	$MgCO_3$	84.32	42.16
Magnesium chloride	$MgCl_2$	95.23	47.62
Magnesium hydroxide (milk of magnesia)	$Mg(OH)_2$	58.34	29.17
Magnesium nitrate	$Mg(NO_3)_2$	148.19	74.10
Magnesium oxide	MgO	40.32	20.16
Magnesium pyrophosphate	$Mg_2P_2O_7$	222.68	
Magnesium sulfate	$MgSO_4$	120.36	60.18
Magnesium sulfate, crystals (Epsom salts)	$MgSO_4.7H_2O$	246.50	123.24
Manganic oxide	Mn_2O_3	157.86	26.31
Manganous oxide	MnO	70.93	35.47
Manganous sulfate	$MnSO_4$	150.99	75.50
Manganous sulfate, crystals	$MnSO_4.4H_2O$	223.05	111.53
Methane	CH_4	16.03	
Molybdic acid	H_2MoO_4	162.02	

TABLE 4.—MOLECULAR AND EQUIVALENT WEIGHTS.—(*Continued*)

Compound	Formula	Molecular weight	Equivalent weight
Nitric acid	HNO_3	63.02	63.02
Oxalic acid	$H_2C_2O_4$	90 02	45.01
Oxalic acid, crystals	$H_2C_2O_4.2H_2O$	126.05	63.02
Oxygen	O_2	32.00	8.00
Phosphoric acid	H_3PO_4	98.04	32.68
Phosphorus pentoxide	P_2O_5	142.05	
Platinic chloride	$PtCl_4$	337.06	84.76
Potassium biniodate	$KIO_3.HIO_3$	389.95	32.49
Potassium bromate	$KBrO_3$	167.02	167.02
Potassium carbonate	K_2CO_3	138.20	69.10
Potassium chloride	KCl	74.56	74.56
Potassium chloroplatinate	K_2PtCl_6	486.17	
Potassium chromate	K_2CrO_4	194.20	
Potassium cyanide	KCN	65.11	65.11
Potassium dichromate	$K_2Cr_2O_7$	294.20	49.04*
Potassium ferricyanide	$K_3Fe(CN)_6$	329.19	
Potassium hydroxide (caustic potash)	KOH	56.11	56.11
Potassium iodide	KI	166 03	166.03
Potassium permanganate	$KMnO_4$	158.03	31.61*
Potassium nitrate (saltpeter)	KNO_3	101.11	101.11
Potassium oxalate, crystals	$K_2C_2O_4.H_2O$	184.22	92.11
Potassium oxide	K_2O	94.20	47.10
Potassium sulfate	K_2SO_4	174.26	87.13
Potassium thiocyanate	KCNS	97.17	97.17
Silicon dioxide (silica)	SiO_2	60.06	
Silver chloride	AgCl	143.34	143.34
Silver chromate	Ag_2CrO_4	331.76	
Silver nitrate	$AgNO_3$	169.89	169.89
Silver nitrite	$AgNO_2$	153.89	76.94
Silver sulfate	Ag_2SO_4	311.82	155.91
Sodium bicarbonate (baking soda)	$NaHCO_3$	84.00	84.00
Sodium carbonate, anhydrous (soda ash)	Na_2CO_3	105.99	53.00
Sodium carbonate (sal soda)	$Na_2CO_3.10H_2O$	287.15	143.58
Sodium chloride (common salt)	NaCl	58.45	58.45
Sodium hydroxide (caustic soda)	NaOH	40.00	40.00
Sodium hypochlorite	NaOCl	74.45	
Sodium nitrate (Chile saltpeter)	$NaNO_3$	85.01	85.01
Sodium oxalate	$Na_2C_2O_4$	134.00	67.00
Sodium phosphate, mono-	NaH_2PO_4	120.04	40.01
Sodium phosphate, di-	Na_2HPO_4	142.02	47.34
Sodium phosphate, tri-	Na_3PO_4	164.01	54.67
Sodium sulfate	Na_2SO_4	142.05	71.03
Sodium sulfate (Glauber's salt)	$Na_2SO_4.10H_2O$	322.21	161.11
Sodium thiosulfate (hypo)	$Na_2S_2O_3$	158.11	158.11
Sodium thiosulfate, crystals	$Na_2S_2O_3.5H_2O$	248.19	248.19
Sulfuric acid (oil of vitriol)	H_2SO_4	98.08	49.04
Sulfurous acid	H_2SO_3	82.08	41.04
Water	H_2O	18.02	9.01

* Oxidation and reduction in acid medium.

TABLE 5.—CHEMICAL EQUATIONS

Neutralization reactions:

$HCl + NaOH = NaCl + H_2O$

$2HCl + Na_2CO_3 = 2NaCl + H_2CO_3$

$H_2SO_4 + 2NaOH = Na_2SO_4 + 2H_2O$

$H_2SO_4 + Na_2CO_3 = Na_2SO_4 + H_2CO_3$

$H_2SO_4 + 2NaHCO_3 = Na_2SO_4 + 2H_2CO_3$

$H_2SO_4 + Ca(HCO_3)_2 = CaSO_4 + 2H_2CO_3$

$H_2SO_4 + CaCO_3 = CaSO_4 + H_2CO_3$

$2NaOH + CO_2 = Na_2CO_3 + H_2O$

$NH_4OH + HCl = NH_4Cl + H_2O$

Double decomposition:

$MgCl_2 + Ca(OH)_2 = Mg(OH)_2 + CaCl_2$

$MgSO_4 + Ca(OH)_2 = Mg(OH)_2 + CaSO_4$

$CaO + H_2O = Ca(OH)_2$

$AgNO_3 + NaCl = AgCl + NaNO_3$

$2AgNO_3 + Na_2CrO_4 = Ag_2CrO_4 + 2NaNO_3$

$K_2CrO_4 + H_2SO_4 = H_2CrO_4 + K_2SO_4$

$H_2CrO_4 = CrO_3 + H_2O$

$FeCl_3 + 3NH_4OH = Fe(OH)_3 + 3NH_4Cl$

$AlCl_3 + 3NH_4OH = Al(OH)_3 + 3NH_4Cl$

$Ca(OH)_2 + (NH_4)_2C_2O_4 = CaC_2O_4 + 2NH_4OH$

$MgCl_2 + NH_4OH + Na_2HPO_4 = MgNH_4PO_4 + 2NaCl + H_2O$

$2MgNH_4PO_4 + \text{heat} = Mg_2P_2O_7 + 2NH_3 + H_2O$

$BaCl_2 + CaSO_4 = BaSO_4 + CaCl_2$

$2KCl + H_2PtCl_6 = K_2PtCl_6 + 2HCl$

$FeCl_3 + 3KCNS = Fe(CNS)_3 + 3KCl$

$MnSO_4 + 2KOH = Mn(OH)_2 + K_2SO_4$

$2(NH_4)_3PO_4 \cdot 12MoO_3 + 46KOH = 2(NH_4)_2HPO_4 + (NH_4)_2MoO_4 + 23K_2MoO_4 + 22H_2O$

$Al_2(SO_4)_3 \cdot 18H_2O + 3CaO = 2Al(OH)_3 + 3CaSO_4 + 15H_2O$

$Al_2(SO_4)_3 \cdot 18H_2O + 3Ca(OH)_2 = 2Al(OH)_3 + 3CaSO_4 + 18H_2O$

$Al_2(SO_4)_3 \cdot 18H_2O + 3Ca(HCO_3)_2 = 2Al(OH)_3 + 3CaSO_4 + 18H_2O + 6CO_2$

$Al_2(SO_4)_3 \cdot 18H_2O + 3Na_2CO_3 = 2Al(OH)_3 + 3Na_2SO_4 + 15H_2O + 3CO_2$

$Ca(OH)_2 + CO_2 = CaCO_3 + H_2O$

$Ca(OH)_2 + Ca(HCO_3)_2 = 2CaCO_3 + 2H_2O$

$Ca(OH)_2 + FeSO_4 \cdot 7H_2O = CaSO_4 + Fe(OH)_2 + 7H_2O$

$Ca(OH)_2 + MgCO_3 = CaCO_3 + Mg(OH)_2$

$Ca(OH)_2 + Mg(HCO_3)_2 = CaCO_3 + MgCO_3 + 2H_2O$

$Ca(OH)_2 + Na_2CO_3 = CaCO_3 + 2NaOH$

$CaCO_3 + CO_2 + H_2O = Ca(HCO_3)_2$

$CaCl_2 + Na_2CO_3 = CaCO_3 + 2NaCl$

$CaSO_4 + Na_2CO_3 = CaCO_3 + Na_2SO_4$

$MgCl_2 + Ca(OH)_2 = Mg(OH)_2 + CaCl_2$

TABLE 5.—CHEMICAL EQUATIONS.—(*Continued*)

$MgSO_4 + Ca(OH)_2 = Mg(OH)_2 + CaSO_4$
$MgCl_2 + Na_2CO_3 = MgCO_3 + 2NaCl$
$MgSO_4 + Na_2CO_3 = MgCO_3 + Na_2SO_4$

Oxidation-reduction reactions:

$K_2Cr_2O_7 + 6KI + 7H_2SO_4 = 4K_2SO_4 + Cr_2(SO_4)_3 + 7H_2O + 3I_2$
$2Na_2S_2O_3 + I_2 = Na_2S_4O_6 + 2NaI$
$2KMnO_4 + 5(NH_4)_2C_2O_4 + 8H_2SO_4 = K_2SO_4 + 2MnSO_4 + 5(NH_4)_2SO_4 + 10CO_2 + 8H_2O$
$2KMnO_4 + 5Na_2C_2O_4 + 8H_2SO_4 = 5Na_2SO_4 + K_2SO + 2MnSO_4 + 10CO_2 + 8H_2O$
$2KMnO_4 + 5CaC_2O_4 + 8H_2SO_4 = K_2SO_4 + 2MnSO_4 + 5CaSO_4 + 10CO_2 + 8H_2O$
$2Mn(OH)_2 + O_2 = 2MnO(OH)_2$
$2KMnO_4 + 10KI + 16HCl = 12KCl + 2MnCl_2 + 8H_2O + 5I_2$
$2KMnO_4 + 5H_2C_2O_4 + 3H_2O = K_2SO_4 + 2MnSO_4 + 10CO_2 + 8H_2O$

TABLE 6.—CONVERSION FACTORS

1 milligram per liter	= 1 part per million
1 kilogram	= 2.205 pounds
1 pound	= 453.6 grams
1 grain per gallon	= 17.12 parts per million
1 grain per gallon	= 142.9 pounds per million gallons
1 part per million	= 0.0584 grain per gallon
1 gallon	= 231 cubic inches
1 cubic foot	= 7.48 gallons
1 cubic foot of water	= 62.4 pounds
1 gallon of water	= 8.34 pounds
1 gallon	= 3.785 liters
1 liter	= 0.2642 gallon
1 liter	= 1.057 quarts
1 liter	= 61.02 cubic inches
1 inch	= 2.54 centimeters
1 centimeter	= 0.3937 inch
1 cubic foot per second	= 646,300 gallons per 24 hours
1 cubic foot per second	= 449 gallons per minute
1,000,000 gallons per 24 hours	= 1.547 cubic feet per second
1,000,000 gallons per 24 hours	= 694 gallons per minute
1 part per million	= 8.34 pounds per million gallons
1 pound per million gallons	= 0.1199 parts per million
1 acre	= 43,560 square feet
1 gram	= 15.432 grains
1 pound	= 7000 grains
1 meter	= 39.37 inches
1 cubic centimeter	= 0.0610 cubic inch
1 cubic inch	= 16.387 cubic centimeters
1 quart	= 0.946 liter
1 gram	= 0.0353 ounce
1 ounce	= 28.3495 grams

Centigrade temperature = (Fahrenheit − 32) × 5/9
Fahrenheit temperature = (centigrade × 9/5) + 32

TABLE 7.—METRIC SYSTEM OF MEASURES AND WEIGHTS

Length	
1 meter	= 10 decimeters (dm.)
1 decimeter	= 10 centimeters (cm.)
1 centimeter	= 10 millimeters (mm.)
Volume	
1 liter (l.)	= 1000 milliliters (ml.)
Mass	
1 kilogram (kg.)	= 1000 grams (gm.)
1 gram	= 1000 milligrams (mg.)

TABLE 8.—CHEMICAL FACTORS*

Known	Unknown	Factor
$AgNO_3$	Cl	0.2087
Al	Al_2O_3	1.8856
Al_2O_3	Al	0.5303
Al_2O_3	$Al_2(SO_4)_3 \cdot 18H_2O$	6.5375
$Al_2(SO_4)_3 \cdot 18H_2O$	Al_2O_3	0.1530
$Al_2(SO_4) \cdot 18H_2O$	CaO	0.2525
$Al_2(SO_4)_3 \cdot 18H_2O$	$Ca(OH)_2$	0.3335
$Al_2(SO_4)_3 \cdot 18H_2O$	Na_2CO_3	0.4776
$BaSO_4$	SO_4	0.4115
Ca	CaO	1.3390
Ca	$CaCO_3$	2.4967
Ca	$Ca(OH)_2$	1.8487
Ca	$Ca(HCO_3)_2$	4.0443
Ca	$CaCl_2$	2.7693
Ca	$CaSO_4$	3.3967
Ca	$KMnO_4$	1.5771
$CaCl_2$	$CaCO_3$	0.902
$CaCl_2$	Na_2CO_3	0.9550
$CaCO_4$	$Ca(OH)_2$	0.7404
$CaCO_3$	CaO	0.5604
$CaCO_3$	Mg	0.2430
$CaCO_3$	CO_2	0.4395
$CaCO_3$	H_2SO_4	0.9796
$CaCO_3$	Na_2CO_3	1.0591
$CaH(CO_3)_2$	$CaCO_3$	0.617
CaO	Ca	0.7149
CaO	$Ca(OH)_2$	1.3208
CaO	$CaCO_3$	1.7843
CaO	CO_2	0.7846
$Ca(OH)_2$	CaO	0.7571
$Ca(OH)_2$	CO_2	0.5938
$Ca(OH)_2$	$CaCO_3$	1.351
$CaSO_4$	Na_2CO_3	0.7786
$CaSO_4$	$CaCO_3$	0.735
CO_2	CaO	1.2750
CO_2	$Ca(OH)_2$	1.6840
CO_2	$CaCO_3$	2.2750
CO_2	NaOH	0.9102
Fe	Fe_2O_3	1.4298
Fe	$FeCl_3$	2.9025
Fe	$FeSO_4 \cdot 7H_2O$	4.9787
Fe	$FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$	7.0224
Fe	$CaCO_3$	1.7941
Fe_2O_3	Fe	0.6994
Fe_2O_3	$FeCl_3$	2.0317
Fe_2O_3	$FeSO_4$	1.9026
$FeSO_4 \cdot 7H_2O$	$FeSO_4$	0.5464
$FeSO_4 \cdot 7H_2O$	Fe	0.2008
$FeSO_4(NH_4)_2SO_4 \cdot 6H_2O$	Fe	0.1424
HCl	Na_2CO_3	1.4534
H_2SO_4	$CaCO_3$	1.0208

TABLE 8.—CHEMICAL FACTORS.*—(*Continued*)

Known	Unknown	Factor
H_2SO_4	CaO	0.5720
H_2SO_4	$Ca(OH)_2$	0.7556
H_2SO_4	Na_2CO_3	1.0809
H_2SO_4	NaOH	0.8168
H_2SO_4	Mg	0.2480
I	O	0.0630
I	$Na_2S_2O_3 \cdot 5H_2O$	1.9553
$K_2Cr_2O_7$	$Na_2S_2O_3 \cdot 5H_2O$	2.5307
$KIO_3 \cdot HIO_3$	$Na_2S_2O_3 \cdot 5H_2O$	7.6371
$KMnO_4$	Ca	0.6341
$KMnO_4$	Mn	0.3476
$KMnO_4$	O	0.2531
K_2PtCl_6	K	0.1608
K_2PtCl_6	KCl	0.3067
K_2PtCl_6	K_2O	0.1937
Mg	$CaCO_3$	4.1151
Mg	$Mg(OH)_2$	2.3960
Mg	CaO	2.3059
Mg	$Ca(OH)_2$	3.0467
Mg	$MgSO_4$	4.9490
Mg	$MgCl_2$	3.9105
MgCl	$CaCO_3$	1.051
$Mg(HCO_3)_2$	$CaCO_3$	0.680
Mg(OH)	$CaCO_3$	1.716
$Mg_2P_2O_7$	Mg	0.2184
$MgSO_4$	$CaCO_3$	0.831
N	NH_4Cl	3.8190
N	NH_3	1.2158
N	NO_2	3.2844
N	NO_3	4.4266
Na	NaCl	2.5417
NaCl	Na	0.3934
Na_2CO_3	H_2SO_4	0.9253
Na_2CO_3	$Al_2(SO_4)_3 \cdot 18H_2O$	2.0959
Na_2CO_3	$CaCO_3$	0.9442
NaOH	H_2SO_4	1.2242
NaOH	CO_2	1.0986
$Na_2S_2O_3 \cdot 5H_2O$	I	0.5114
$Na_2S_2O_3 \cdot 5H_2O$	O	0.003223
$Na_2S_2O_3 \cdot 5H_2O$	$K_2Cr_2O_7$	0.3951
$Na_2S_2O_3 \cdot 5H_2O$	$KIO_3 \cdot HIO_3$	0.1309
NH_3	N	0.8225
$(NH_4)_2C_2O_4 \cdot H_2O$	$KMnO_4$	0.4448
NO_2	N	0.3045
NO_3	N	0.2259
O	$Na_2S_2O_3 \cdot 5H_2O$	31.0238
O	I	15.8665
O	$KMnO_4$	3.9507
Pt	K_2O	0.4825

* The above factors are derived from the equivalent weights of the various elements, radicals and compounds. To change from the weight of the known compound to its equivalent of the unknown multiply the weight of the known by the factor.

TABLE 9.—SOLUBILITY OF OXYGEN IN FRESH WATER

Temperature		Dissolved oxygen, p.p.m.
°C.	°F.	
0	32.0	14.62
1	33.8	14.23
2	35.6	13.84
3	37.4	13.48
4	39.2	13.13
5	41.0	12.80
6	42.8	12.48
7	44.6	12.17
8	46.4	11.87
9	48.2	11.59
10	50.0	11.33
11	51.8	11.08
12	53.6	10.83
13	55.4	10.60
14	57.2	10.37
15	59.0	10.15
16	60.8	9.95
17	62.6	9.74
18	64.4	9.54
19	66.2	9.35
20	68.0	9.17
21	69.8	8.99
22	71.6	8.83
23	73.4	8.68
24	75.2	8.53
25	77.0	8.38
26	78.8	8.22
27	80.6	8.07
28	82.4	7.92
29	84.2	7.77
30	86.0	7.63

PART II

BACTERIOLOGICAL ANALYSIS OF WATER AND SEWAGE

SECTION I

SAMPLING

Bottles.

Samples of water or sewage for bacterial analysis should be collected in suitable bottles that have been carefully cleaned, rinsed in clean water (preferably distilled water) and sterilized according to directions in Sec. III, page 238.

Two kinds of bottles may be used, (1) clean sterile bottles, and (2) clean sterile sodium thiosulfate-treated bottles (page 241). Water or sewage effluents containing residual chlorine should always be collected in sodium thiosulfate-treated bottles. All other samples may be collected in clean sterile bottles, although the use of a sodium thiosulfate-treated bottle is not objectionable. (In our laboratory all sample bottles contain sodium thiosulfate. This avoids confusion with two types of bottles.)

The most desirable type bottle is a 4- or 8-oz. salt-mouth, ground-glass-stoppered bottle. A 4-oz. bottle is most satisfactory for samples wherein the portions tested do not total in excess of 60 ml.

The stopper and neck of the sample bottle should be covered to protect against dust and handling contacts. A suitable-sized square of brown manila wrapping paper pressed over the stopper and neck, secured by a rubber band or string, has been found very satisfactory.

Collection of Samples.

In collecting samples, extreme care should be exercised to avoid contaminating parts of the bottle coming in contact with the water. The stopper should be handled without removal of the protective cover. Bottles should be filled to three-quarters of their capacity. If samples are collected in sodium thiosulfate-treated bottles, care must be exercised not to rinse the bottle and lose the sodium thiosulfate.

1. *Samples from Water Systems.*

When samples are collected from a water system, the tap and piping should be thoroughly flushed before collecting the sample. The extent of the flushing will depend upon the amount of piping in which the water may have been standing and the time since water was drawn from the tap. It is advisable to collect the water for sampling from taps that are in frequent use. Avoid sampling from taps which are subject to contamination, for example, taps in lavatories. Flaming taps by burning paper or matches beneath them has no value. If flaming is used, sufficient heat should be applied to heat the tap to boiling-water temperature. If the surfaces of the tap are dry there is little danger of contaminating the sample, provided the water is allowed to flow several minutes. Avoid sampling from wet taps. Never sample from rubber hose or any other temporary attachment fastened to the tap.

2. *Samples from Pools, Lakes and Rivers.*

If the samples are collected from pools, lakes or rivers, care should be exercised to obtain the sample from a point which represents average conditions of the supply.

In all cases where samples are collected from standing water, remove the stopper aseptically and plunge the bottle beneath the surface, mouth down, to a depth of 3 in. or more. Fill the bottle at this depth, moving the bottle forward away from the hand so that water which has come in contact with the hand will not enter the bottle. Discard about a quarter of the water and replace the stopper.

Pools.—In a swimming pool, the sample should be collected at the side of the pool at a point near the deepest part. Samples should be collected during periods of use, preferably at the time of the heaviest bathing load of the day. If samples collected at such times are satisfactory, it is reasonable to expect that samples collected at times of lesser load will also be satisfactory. Samples should never be taken in the absence of bathers. Samples should be collected in sodium thiosulfate-treated bottles. Chlorine residuals should be made preferably at the pool side but if test must be made at the laboratory, samples for this purpose should be collected in bottles without sodium thiosulfate.

Lakes.—Lake samples should never be collected from the shore. They should be collected from a boat at a distance from the shore of at least 25 ft. or more, depending upon the depth. Arbitrarily the water should have a depth of at least 4 ft. and preferably more at the sampling point.

Rivers.—In a stream, samples should represent the flowing water and not stagnant pools. In a straight stretch of the river, samples may be collected from the banks but at a distance of at least 4 ft. In a meandering stream the samples should be collected near the center at the point of greatest depth.

3. *Samples of Sewage and Sewage Effluents.*

The sample should always be collected directly from the sewage or sewage effluent into the sample bottle. Never collect the sample by means of a common collecting container.

When composite samples are desired, samples should be collected in sterile containers. They may be emptied into a larger sterile bottle, large enough to hold the composite sample. The composite sample bottle should be stored at a temperature of 6° to 10°C. during the period of collection.

Transportation and Storage of Samples.

Owing to biological changes that may occur in the sample of water or sewage, all samples should be tested as soon as possible. In warm weather, if the transportation period exceeds more than 1 hr., the sample should be iced.

The time of transportation and storage should not exceed 6 hr. for impure waters and not more than 12 hr. for relatively pure waters. Samples should be stored at a temperature between 6 and 10°C.

This does not mean, however, that all samples exceeding these storage periods should be discarded, as occasionally it is necessary to store samples overnight. In general, the changes incurred by storage for 12 to 18 hr. are slight. *Samples stored longer than 24 hr. should be discarded or the results obtained should be judged accordingly.*

It is necessary that information on the source of water accompany the sample to the laboratory. This information may be very helpful in interpreting the laboratory findings. A report sheet used by the Michigan Department of Health is presented.

Sample No. F28—2-43—20M

Copies..... Reported..... Received.....

DESCRIPTION OF SOURCE AND RESULTS OF ANALYSIS OF SAMPLE OF WATER

Answer all numbered questions—circle words that apply—USE SOFT LEAD PENCIL

1. Collected by.....
2. Collected at.....
   (street address, well number, etc.)
3. Collection: date..... hour.....
   (month—day—year)
4. Date of last sample.....
   (month—day—year)
5. *Source of supply:* River, lake, spring, well.
6. Kind of supply: private, public, school, municipal.
7. Sampling point: pump, pressure tank, tap, reservoir, fire hydrant, fountain, or.....
8. *Pollution within 75 feet of well:* privy.....ft., sewer.....ft., septic tank.....ft., seepage pit.....ft., cesspool.....ft., barnyard..... ft., none (Give distances in space provided.)
9. Repairs to pump or well in last two months: none, leathers, rods, new pump, cleaned well, or.....
10. Reason for analysis.....
11. WELL: Diameter..... Depth.....
    Age..... Flowing: yes, no.
12. Pump Rods: wood, metal, none.

HAND PUMP WELL

13. Platform: Wood, concrete, none.
14. Water-tight connection between pump base and platform: yes, no.
15. Has the well a frost-pit: yes, no.
16. Does water ever stand in pit: yes, no.
17. *Material in outside casing:* iron pipe, brick, stone, crock, concrete.
18. Top of spout: open, closed.
19. Does pump require priming: yes, no.

POWER PUMP WELL

20. Location of pump: above ground, in pit, in basement, directly above casing, offset from casing.
21. Kind of pump: deep cylinder, turbine, shallow suction, ejector.
22. Top of casing in respect to floor: below, flush, above.
23. Water-tight connection between pump or drop-pipe and casing: yes, no.

Person to whom results should be sent. Name:.....
Address:.....

24. County.....
25. Township.....
26. Post office.....
27. Name of owner.....

BACTERIOLOGICAL ANALYSIS
Not to be filled in by sender

Bacteria per ml.
Agar 37°C. 24 hrs.

Hours	Gas in lactose broth									
48										
24										
Sample in milliliters					10	1	.1	.01	.001	.0001

COLI-AEROGENES GROUP

Confirmation: E.M.B. L.B. B.G.B.
Index: per 100 ml.

Hardness pH
Chlorides Iron

Miscroscopic Examination

C. C. Young
Examiner—*Director*, Bureau of Laboratories

FIG. 3.

In Cooperation With U. S. Public Health Service

MICHIGAN DEPARTMENT OF HEALTH

Bureau of Laboratories

Lansing Houghton Powers Grand Rapids

DIRECTIONS FOR COLLECTING AND SENDING WATER SAMPLES FOR BACTERIOLOGICAL ANALYSIS:

1. Bottles sent out from the laboratory are always sterilized and require no further treatment.

2. To collect a sample, remove glass stopper with paper attached so that hands do not come in contact with stopper or mouth of bottle. Do not attempt to remove strip of paper under stopper; allow it to be washed into the bottle with the water. This paper is sterilized and will in no way affect the results of analysis.

3. When collecting water from a pump or faucet, allow it to run ... tap is seldom necessary. If sample must be collected ... blank. When collecting from a lake or stream, cut st ... Remove stopper and hold it by paper cover. Collect s... forward or upstream and submerged about one foot.

4. Never fill the bottle completely; always leave a small air space. Insert the stopper immediately, fastening the paper cover down with rubber band or string. Do not seal with wax or other material.

5. Fill out the information blank as completely as possible and send it with the sample by mail or prepaid express to the Laboratory of the Michigan Department of Health either at Lansing, Powers, Houghton or Grand Rapids, whichever is most convenient. As soon as the analysis is completed a report will be forwarded with an opinion on its suitability for drinking purposes.

6. Water samples should be collected and mailed at once so as to reach the laboratory as soon as possible.

GUIDE TO CONSTRUCTION OF WATER WELLS FOR HOMES

Isolation from sewage. A well for a home should be at least 50 feet from all sources of sewage pollution, such as sewers, septic tanks, cesspools, barnyards, etc.

Surface drainage away from well. The ground surface should be graded to permit a slope away from the well in all directions.

Casing water-tight. Metal casings should be water-tight throughout their entire length and the wall of a dug well should be water-tight for at least the top 10 feet.

Top of casing above platform or floor. For wells equipped with hand pumps and less than 2 inches in diameter, the casing should extend above the concrete platform and be connected to the pump by a threaded or flanged joint; for wells 2 inches in diameter and larger the casing should extend at least one inch above the concrete platform and into the pump base.

The casing of wells equipped with power pumps should extend at least 12 inches above the floor.

The wall of a dug well should extend at least 4 inches above the floor or the original ground surface.

Diameter of casing. It is preferable to use a 2 or 2½ inch, or larger, casing to permit the cylinder to be submerged in the water in the well. Such wells need no frost pit nor require priming.

Water-tight connection at top of casing. If it is possible to see or pour a liquid into the well after the pump is set, the water supply is not properly protected.

Depth of well. It is recommended that a well be at least 25 feet deep. No well should be less than 10 feet deep Additional protection may be secured by submerging the top of the screen at least 5 feet in the ground water.

Concrete platform or floor. Wells with hand pumps should have water-tight reinforced concrete platforms at least 4 inches thick and four feet square. The top of the platform should slope from the well to the outside edges. Well houses should have a reinforced concrete floor sloped away from the well and drained by gravity to the surface of the ground.

Pumps. A deep cylinder pump with a separate drop pipe is recommended.

The shallow suction pump may be used when the water in the well is not more than 15 to 20 feet below the cylinder. Shallow suction pumps frequently require priming.

Ejector pumps are satisfactory from a health viewpoint.

The base of a hand pump should be of the solid one-piece recessed type, cast as a part of or threaded to the pump column. Split and adjustable bases are not recommended. The top of the pump should not be slotted but should be equipped with a stuffing box through which the pump rod works. Pumps with open spouts should not be used.

Well Pits. They should not be used.

For additional information on the construction of well water supplies for homes, request a copy of Bulletin No. 14, Well Water Supplies, from your county health department or the Michigan Department of Health, Lansing. Copies are available free. Consult your county or city health department if you have a specific well water supply problem regarding which you would like some advice.

FIG. 4.

SECTION II

PROCEDURES

This section is divided into three divisions. Division I gives methods of procedure for making various bacteriological determinations and is accompanied by detailed explanations of all steps and technique required. Division II gives only the steps involved in each determination with omission of all general instructions and explanations. It is intended that Division II will serve as an outline of procedure and be used only after the analyst is familiar with the explanations and technique given in Division I. Division III gives methods of staining.

DIVISION I

PROCEDURES WITH DETAILED EXPLANATIONS

1. Shake the sample of water vigorously 25 times.
2. Flame the mouth of the bottle, unless the stopper is protected by a covering. Remove the stopper.
3. By means of sterile pipettes, transfer (plant) suitable amounts of the sample or its dilutions to Durham fermentation tubes containing nutrient lactose broth (page 243). The fermentation tubes should contain sufficient broth of a concentration such that when the water portion has been added the concentration of peptone in the resulting dilution will be between 0.3 and 0.5 per cent. (Flaming the mouth of the fermentation tube just previous to adding the sample is a good precaution against contamination.)

The amounts of water to be tested for colon index and the number of tubes used depend upon the kind of water tested and the purpose of the test:

Potable Waters.—If the water represents a finished water from a purification plant or well, five 10-ml. portions should be tested. Transfer 10 ml. of water aseptically to each of five Durham fermentation tubes containing either 30 ml. of nutrient lactose broth or 8 to

10 ml. of double-strength nutrient lactose broth. In addition to the five 10-ml. portions tested transfer a 1-ml. portion to a Durham fermentation tube containing approximately 8 ml. of nutrient lactose broth.

Raw Water, Unfinished Water or Sewage.—Dilutions must be used if the water is unpotable and is known to contain considerable bacteria. The dilutions are made by transferring, with a sterile pipette, a 10-ml. portion of the sample to a dilution flask containing 90 ml. of sterile water (page 251), making a 1 to 10 dilution. After shaking vigorously 25 times, a 10-ml. portion of the 1 to 10 dilution is transferred, using a sterile pipette, to a second dilution flask containing 90 ml. of sterile water, making a 1 to 100 dilution. After shaking 25 times, a 10-ml. portion of the 1 to 100 dilution is transferred with a sterile pipette to another 90-ml. portion of sterile water, making a dilution of 1 to 1,000. This process is continued until any desired dilution is obtained.

Starting with the greatest dilution and using a sterile pipette, transfer a 1-ml. portion to a Durham fermentation tube containing approximately 8 ml. of nutrient lactose broth. Using the same pipette treat each dilution in the same manner, always proceeding from a higher dilution to the next lower. The dilutions planted depend upon the density of the coliform group in the water. In order to arrive at the correct number of dilutions it is necessary to plant a sufficient number so that the greatest dilution is negative. For example, suppose the water tested has a colon index of 10,000. A 1-ml. portion and dilutions of 1 to 10, 1 to 100 and 1 to 1,000 should be made. Coliform organisms should appear in all the tubes except the 1 to 1,000 dilution. Or suppose a sample of sewage with a colon index of 10,000,000 was tested. The following minimum dilutions should be planted: 1 to 10,000, 1 to 100,000 and 1 to 1,000,000.

4. At the same time that the fermentation tubes are seeded, agar plates should be made.

   a. Using sterile pipettes, place portions of the sample or its dilutions in sterile Petri dishes.

   The amounts of water planted for bacteria counts, like the colon index, depend upon the kind of water to be tested and the purpose of the test.

   Potable Waters.—Two plates of 1 ml. each should be made.

   Raw Waters, Unfinished Water or Sewage.—Dilutions must be used in water or sewage with total bacteria counts in excess of 100 bacteria per milliliter. As stated in Sec. IV, page 261, a desirable plate contains from 30 to 300 colonies. If the bacteria count expected is approximately 10,000 bacteria per milliliter, three plates should be made, using dilutions of 1 to 10, 1 to 100 and 1 to 1,000.

b. Melt tubes or flasks of nutrient agar (page 246) in the steamer or in a container of boiling water and cool by placing in a water bath at 40 to 50°C. Avoid shaking agar as air bubbles are introduced into the agar by violent disturbance. Immediately after water portions or dilutions of the sample have been placed in the Petri dishes, lift the edge of the cover of the Petri dishes and add, aseptically, approximately 8 to 10 ml. of nutrient agar to each. Rotate the dish immediately to mix the sample with the agar and insure an even distribution of the bacteria over the entire surface of the Petri dish. Allow the agar to harden on a level surface and then invert the plates.

5. Incubate the agar plates and the Durham fermentation tubes at 37°C. (It is very important that the temperature of the incubator be maintained at 37°C. Higher temperatures are unfavorable to gas formation and, contrariwise, temperatures below 37°C. favor gas production. Temperatures should be taken on the shelves on which tubes and plates are incubated.)
6. At the end of 24-hr. incubation at 37°C., count the agar, plate colonies (page 261), examine the fermentation tubes (page 255) and record results as follows:
   a. Absence of gas. If no gas has been produced at the end of 24 hr., reincubate for an additional 24 hr. If gas is still absent, the test is considered negative.
   b. Formation of gas. When large volumes of gas (10 per cent or more) are produced, in most cases the causative organisms are members of the coliform group. For this reason 10 per cent or more gas is positive presumptive evidence of the coliform group. If the gas occupies less than 10 per cent of the inverted vial, it generally is caused by the coliform group but because small amounts of gas may be produced by other bacteria this test should be considered doubtful.

Interpretation of gas formation in (*b*).

Raw Waters.—In the routine testing of a raw-water supply where the presence of the coliform group has been previously consistently confirmed, it is not necessary to carry the test any further.

In this case gas may be considered to indicate the presence of the coliform organisms.

Sewage.—In the routine testing of a sewage or sewage effluent, except chlorinated effluents, it is not necessary to carry the test any further.

Swimming Pools, Finished Waters and Chlorinated Sewage Effluents.—All tubes showing gas in (*b*) should be carried through a completed test, as gas production by organisms other than the coliform group may be responsible.

7. Samples failing to show gas or showing only a small bubble in the fermentation tubes should be incubated another 24 hr. at 37°C. If gas appears in these tubes at the end of the 48 hr., they should be carried through a confirmatory test, except as indicated above, for raw waters and sewage. Two options for carrying through the confirmatory test and their suitability for different waters are given below.

Option A or B may be used for:

1. *Wells, lakes, springs,* etc. Where single samples are tested and are not routinely checked.

Option B may be selected preferably for the following types of water and sewage:

2. *Finished waters.* In routine laboratories where a finished water is examined regularly.
3. *Swimming-pool waters.* In routine testing of swimming-pool water, Option B, using brilliant green bile broth, has been found satisfactory.
4. *Sewage and sewage effluents.* In the routine testing of sewage and sewage effluents, chlorinated and nonchlorinated.
5. *Raw waters* (unpotable). In routine examination of unpotable raw waters.

Option A.

8*a*. Partially confirmed test. Streak eosin-methylene blue agar of Endo's medium plates (page 249) from tubes showing gas in the presumptive test, in such a manner that discrete colonies are produced. (This should be done preferably as soon as gas appears.) All tubes that show gas from finished waters, swimming pools and chlorinated sewage effluents should be streaked. The following procedure for streaking plates is suggested:

Bend the tip of a platinum needle at an oblique angle. The distance from the heel of the angle to the tip should be approximately 4 mm. After flaming the needle, dip the bent part into the fermentation tube to be examined. Draw the needle across the surface of the agar plate in such a manner that a strip approximately 4 mm. wide is smeared. At the end of the streak, lift the needle and repeat, being careful not to overlap the first streak. This may be repeated until the entire surface of the plate has been covered. By trial, the number of streaks necessary to obtain discrete colonies may easily be determined.

9*a*. Incubate all plates at 37°C. for 18 to 24 hr. The colonies that appear on the plates should be recorded as follows:

(*a*). Typical colonies.

Eosin-methylene blue agar. The typical colony of the *Escherichia* group (colon group) is characterized by bluish-black, small, shiny, entire, convex colonies with metallic sheen. The typical *Aerobacter* (Aerogenes group) colonies are characterized by spreading, mushy, flat, dull colonies of a brownish color, particularly at the center, without a metallic sheen. On confirmation these colonies generally prove to be members of the coliform group.

Endo's medium. The typical colony of the *Escherichia* group is characterized by a reddish-black color, small, entire, convex colonies with a metallic sheen. It is difficult with this medium to differentiate the *Aerobacter* group from colonies other than those of the *Escherichia* group.

(*b*). Atypical colonies.

If colonies fail to appear in 24 hr. reincubate for an additional 24 hr. All colonies that appear on the plates other than the typical colonies should be regarded as atypical.

10*a*. In the case of typical or atypical colonies found, transfer at least two discrete colonies to lactose broth fermentation tubes (page 243) and nutrient agar slants (page 247), seeding corresponding tubes from the same colony.

11*a*. Incubate at 37°C. for 48 hr. If gas formation does not occur within 48 hr., the test is considered as negative.

12*a*. If the culture in the fermentation tube shows gas, the corresponding agar slant should be examined microscopi-

cally for spores. Examination for spore formation should not be made on cultures less than 24 hr. old.

Interpretation of Step Nos. 11*a* and 12*a*.

If the lactose broth shows gas formation and the corresponding agar slant reveals microscopically Gram-negative nonsporeforming bacilli, the test is complete and the presence of members of the coliform group has been demonstrated.

If the fermentation tube fails to show gas formation or the corresponding agar slant culture shows a Gram-positive organism or a sporeforming bacillus, the test is complete and a negative test is obtained.

If facilities are lacking for microscopic examinations the following may be substituted:

Instead of seeding an agar slant, seeding may be made into formate-ricinoleate broth (page 246). This medium prevents the growth of sporeforming bacteria. If gas is produced in the lactose broth but not in the formate-ricinoleate broth, the organisms are aerobic sporeformers of no sanitary significance.

Option B.

8*b*. Make transfers from all tubes showing gas in the presumptive test to one of the following mediums. (This should be done, preferably, as soon as gas appears.)

(1) Brilliant green bile broth (page 244)
(2) Fuchsin-lactose broth (page 244)
(3) Formate-ricinoleate broth (page 246)
(4) Crystal violet lactose broth (page 244)

The transfer should be made by carrying a loopful of material from the fermentation tube to the selected medium. *In no instance should a straight needle be used.* The loop should be at least 3 mm. inside diameter and made of 21-gauge platinum or Nichrome wire. A sterile capillary tube or pipette may be used if larger quantities of the medium are desired.

The medium selected for routine use should be ascertained for each laboratory by repeated tests using all the mediums listed. The most consistent medium should be adopted as standard for each laboratory. Past experience in many laboratories has demonstrated that brilliant

green bile broth is the most satisfactory. It is suggested that this medium be used.

9*b*. Incubate the tubes at 37°C. for 48 hr.

10*b*. (*a*). If after 48-hr. incubation gas formation does not occur, members of the coliform group are absent and the test may be considered complete and the results reported as negative.

(*b*). If gas appears in 48-hr. incubation, members of the coliform group are present and the test may be considered as complete and the results reported as positive. If the analyst desires a more careful check, he may continue the test by carrying through Option A at this point.

DIVISION II

OUTLINE OF PROCEDURES

Routine Examination for Potable Waters.

1. Plant one 1-ml. and five 10-ml. portions of the sample in nutrient lactose broth (page 243). At the same time plant two 1-ml. samples in agar (page 247) plates for total count.
2. Incubate 24 hr. at 37°C.
3. Make bacteria counts from plates and observe gas production.
4. If gas appears in the fermentation tubes, follow procedure given in Option A or B below.
5. If no gas appears, reincubate an additional 24 hr.
6. If gas appears in 48 hr., follow procedure given in Option A or B below. If no gas appears, the test is negative.

Option A.

7*a*. Smear eosin-methylene blue agar (page 248) or Endo's medium (page 249) from two or more tubes showing gas.
8*a*. Incubate 18 to 24 hr. at 37°C.
9*a*. Transfer typical or atypical colonies to nutrient lactose broth (page 243) and agar slant.
10*a*. Incubate at 37°C. for 24 hr.
11*a*. Positive gas test in lactose broth and Gram-negative non-sporeforming bacillus indicate the presence of the coliform group.

Option B.

7*b*. Transfer a loopful from all positive tubes to one of the following mediums:

(1) Brilliant green bile broth (page 244)
(2) Fuchsin broth (page 244)
(3) Formate-ricinoleate broth (page 246)
(4) Crystal violet broth (page 244)

8*b*. Incubate 48 hr. at 37°C.
9*b*. Gas production indicates the presence of the coliform organisms, whereas no gas indicates the absence of these organisms.

Routine Examination of Unpotable Waters.

1. Plant decimal dilutions of the sample into nutrient lactose broth (page 243) so that the greatest dilution is negative. At the same time plant dilutions in agar (page 247) plates for total count.
2. Incubate 24 hr. at 37°C.
3. Make bacteria counts from plates and observe gas production.
4. Record all tubes showing gas as positive.
5. Calculate the colon index from the results obtained in Step No. 4.
6. If no gas is formed in 24 hr., treat the sample as under Potable Waters beginning at Step No. 5.

Routine Examination of Swimming-pool Waters.

1. Plant one 1-ml. and five 10-ml. portions of the sample in nutrient lactose broth (page 243). At the same time plant two 1-ml. portions in agar (page 247) plates for total counts.
2. Incubate 24 hr. at 37°C.
3. Make bacteria counts from plates and observe gas production.
4. If gas appears, proceed to Step No. 7.
5. If no gas appears, reincubate 24 hr. at 37°C.
6. If gas appears in 48 hr., proceed to Step No. 7. If no gas appears, the test is negative.
7. Transplant a loopful of material from each tube showing gas to brilliant green bile broth (page 244). (Option A may be followed at this point if desired.)
8. Incubate tubes 48 hr. at 37°C. Record all tubes showing gas as positive.
9. Calculate the colon index (page 258) from the results obtained in Step No. 8.

Examination of Sewage and Sewage Effluents.

1. Plant decimal dilutions of the sample into nutrient lactose broth (page 243) so that the greatest dilution is negative. At

the same time plant suitable dilutions in agar (page 247) plates for total counts.
2. Incubate 24 hr. at 37°C.
3. Make bacteria counts from plates and observe gas production.
4. Record as positive all tubes showing gas.
5. Calculate the colon index (page 258) from the results obtained in Step No. 4.

Examination of Chlorinated Sewage Effluents.

1. Plant decimal dilutions of the sample into nutrient lactose broth (page 243) so that the greatest dilution is negative. At the same time plant dilutions in agar (page 247) plates for total counts.
2. Incubate 24 hr. at 37°C.
3. Make bacteria counts from plates and observe gas production.
4. If gas appears, proceed to Step No. 7.
5. If no gas is formed, reincubate at 37°C.
6. If gas appears in 48 hr., proceed to Step No. 7. If no gas appears, the test is negative.
7. Transplant a loopful of material, from the tube showing gas from the greatest dilution of the sample, to brilliant green bile broth (page 244). (Option A may be followed at this point, if desired.)
8. Incubate 48 hr. at 37°C. Record as positive all tubes showing gas.
9. Calculate the colon index (page 258) from the results obtained in Step No. 8.

An Optional Method for Sewage and Sewage Effluents (Chlorinated and Nonchlorinated).

1. Plant decimal dilutions of the sample into brilliant green bile broth (page 244) so that the greatest dilution is negative. At the same time, plant suitable dilutions in agar (page 247) plates, for total counts.
2. Incubate the agar plates 24 hr. and make bacteria counts.
3. Incubate the tubes 48 hr. and record all tubes showing gas as positive. If no gas appears, the test is negative.
4. Calculate the colon index (page 258) from the results obtained in Step No. 3.

DIVISION III

METHODS OF STAINING

Spore Stain.*

1. Flame a glass slide by passing it through the flame.
2. Place one loopful of distilled water on the slide.
3. Touch the growth on the agar-slant culture lightly with a sterilized needle and transfer a small amount of the material to the distilled water on the slide.
4. Spread the drop over an area of approximately 4 sq. cm.
5. Allow to dry in the air.
6. Fix the preparation on the slide by passing the slide, specimen side up, through the flame until the underside is uncomfortable to the fingers.
7. Flood the slide with 5 per cent aqueous malachite green (page 252).
8. Warm over a flame until steam appears. Continue the steaming for 10 min., keeping the slide flooded by the addition of fresh staining fluid.
9. Wash for ½ min. in running water.
10. Counterstain by flooding the slide with 0.5 per cent safranin O (page 252). Allow to act for 1 min.
11. Wash in running water.
12. Dry slide and examine.

Note.—The spores are stained green, the rest of the cells red.

Gram's Method of Staining.†

1. Flame a glass slide by passing it through a flame.
2. Place one loopful of distilled water on the slide.

* Schaeffer, A. B., and McD. Fulton, A Simplified Method of Staining Endospores, *Science*, vol. 77, p. 194, 1933.

† Burke, Victor, Notes on the Gram Stain with Description of a New Method, *Journal of Bacteriology*, vol. 7, p. 159, 1922.

3. Touch the growth on the agar-slant culture lightly with a sterilized needle and transfer a very small amount of the material to the distilled water on the slide. Only enough of the material should be transferred to make the water slightly cloudy.
4. Spread the drop over an area of approximately 4 sq. cm.
5. Allow to dry in the air.
6. Fix the preparation on the slide by passing the slide, specimen side up, through the flame until the underside is uncomfortable to the fingers.
7. Flood the slide with a 1 per cent aqueous solution of methyl violet. Mix 5 to 8 drops of a 5 per cent solution of sodium bicarbonate (page 251) with the methyl violet solution. Allow to stand for 2 to 3 min.
8. Flush off the excess stain with iodine solution (page 251). Cover with more iodine solution and let stand 1 min.
9. Wash in water and remove practically all of the water by blotting, but do not allow the smear to become dry.
10. Decolorize with acetone-ether (page 252) until the decolorizer flows from the slide almost uncolored.
11. Wash with water and blot or air-dry.
12. Counterstain, using a 2 per cent aqueous solution of safranin O (page 251).
13. Examine with the 2-mm. oil-immersion objective. A Gram-positive organism stains blue and a Gram-negative organism stains red.

SECTION III

PREPARATION OF MEDIUMS AND SOLUTIONS

DIVISION I

CLEANING, STERILIZATION, FILTRATION AND pH

Preparation of Glassware.

Cleaning.

All glassware used in the cultivation of microorganisms must be chemically clean. The presence of undesirable organic and inorganic substances may prevent the development of the culture by altering the nutrients, injuring the normal metabolism of the organism or inhibiting growth and reproduction.

New glassware may contain sufficient alkali to change materially the reactions of the nutrient medium used and thus prevent microbial growth. All new glassware, particularly that made of soft glass, should be treated with acid solutions to neutralize and remove excess alkali. Chromic acid cleaning solution (page 120) is especially valuable for removing traces of oxidizable organic matter and neutralizing free alkali on the glassware. This solution contains sufficient sulfuric acid to destroy fabrics and bristles of brushes and corrodes metal quickly. For this reason neither cloths nor brushes should be used as an aid to the cleaning solution. Extraneous matter should be removed by means of detergents and brushing before treating with cleaning solutions.

The cleaning of glassware also may be accomplished by various soaps and detergents. Any agent that tends to remove oils and organic soil from the surface of the glassware helps in the cleaning process. Such detergents as trisodium phosphate, sodium metasilicate, sodium carbonate, alone or in combination with sodium hydroxide, are commonly used. The addition of sodium metaphosphate, which removes lime film from glassware, improves the detergent action.

After glassware has been washed free from all chemicals, it should be rinsed in distilled water to remove all chemicals that might be deposited by the evaporation of the water from the surfaces of the glassware. The glassware should be allowed to drain and dry, then it may be prepared for sterilization.

Preparing for Sterilization.

Test Tubes and Flasks.

Test tubes are plugged with nonabsorbent cotton. Test tubes may be plugged with cotton and sterilized prior to filling with nutrient medium, but tubes destined for testing water may be plugged with cotton after filling with medium.

To plug a test tube, grasp a piece of cotton of appropriate size with a pair of forceps and insert it into the mouth of the test tube. The plug should project into the test tube about 3 or 4 cm. It should project out of the mouth of the tube about 1.5 cm., an amount that can easily be grasped by the fingers for the removal of the plug. The plug should be firm; not so tight that it is difficult to remove but not so loose that it is removed without resistance.

Plugs for flasks should always be rolled, that is, a strip of cotton can be rolled and then inserted into the mouth of the flask. Such plugs are stable and may be used several times.

Petri Dishes.

Petri dishes are wrapped separately in paper and tied in bundles of three. One sheet of newspaper makes four papers of proper size. Special Petri dish containers are frequently used.

Sterilization.

Two processes of sterilization are used in the bacteriological laboratory, namely, the dry-heat and the moist-heat processes.

Dry-heat Sterilization.

The factors responsible for the death of bacteria in dry-heat sterilization are desiccation and coagulation. The protoplasm of bacteria contains approximately 85 per cent moisture. As this moisture is reduced, many bacteria die. When the moisture

is decreased to minimum content, most bacteria die, particularly if the moisture is removed rapidly, as it would be when heat is applied. Because the bacterial protoplasm is made up, in part, of albuminous material, coagulation of this material would cause the death of the bacterial cells. The coagulation temperature of albumin varies with the moisture content. The following table gives the coagulation temperature of albumin with various moisture contents:

Egg albumin + 50 per cent moisture coagulates at 56°C.
Egg albumin + 25 per cent moisture coagulates at 74 to 80°C.
Egg albumin + 18 per cent moisture coagulates at 60 to 90°C.
Egg albumin + 6 per cent moisture coagulates at 145°C.
Egg albumin + 0 per cent moisture coagulates at 160 to 175°C.

Inasmuch as the spores of bacteria contain only a small amount of moisture their coagulation temperatures are quite high compared with those for vegetative cells.

Glassware used for culturing bacteria from waters will be contaminated with many kinds of bacteria; many of them spore-forming types. For this reason, all glassware sterilized by dry heat must reach a heat higher than the coagulation temperature of dry albumin to ensure sterilization. Dry-heat sterilization should be done at a temperature of 160 to 180°C. At the lower limits, a considerably longer time should be used. In general, 160°C. for 1 hr. or 180°C. for 10 min. will effect sterilization.

Dry sterilization should be used for pipettes, Petri dishes, test tubes, sample bottles and other dry apparatus that will stand high temperatures without injury. All glassware must be chemically clean and dry or contain only traces of alcohol before sterilization, otherwise sterilization cannot be accomplished. If considerable moisture is present, all the moisture may not evaporate during the heating, and as a result such moist glassware will not attain a temperature in excess of 100°C. Because the heat in the hot-air oven reaches the center by convection currents and because the dead air spaces in the glassware are poor conductors of heat, it is necessary to extend the time of heating according to the amount of glassware and the size of the oven. In an oven having a useful space of 8 cu. ft. filled with glassware, the period of heating at 180°C. should be

at least 2 hr. in order to insure a temperature of 180°C. for 10 min. at the center of the oven.

Petri dishes and pipettes should be placed in suitable containers for sterilization to protect them against contamination after removal from the sterilizing oven.

Moist-heat Sterilization.

The heating of nutrient mediums by boiling or by exposure to flowing steam at a temperature of 100°C. is not a reliable means of sterilization even though a prolonged period of exposure is used. Many bacterial spores, owing to their low water content, may survive one heating at 100°C. By repeated heatings at 100°C., at intervals of 24 hr., these spores may be weakened sufficiently to effect sterilization. This process is called the "Tyndal method" of sterilization. In practice, where only 100°C. moist heat is available, sterilization may be obtained by heating for 15 to 30 min. (depending upon the quantity and the nature of the material to be sterilized) for 3 successive days.

Moist-heat sterilization by steam under pressure is a better method of sterilization. When steam is placed under pressure in an autoclave, the following temperatures may be obtained:

Temperature °C.	Gauge pressure, lb. per sq. in.	Total pressure, lb. per sq. in.
100	0	14.7
108	5	19.4
115	10	24.5
121	15	29.7
126	20	34.7

At temperatures of 115 to 120°C., mediums and other materials can be effectively sterilized in 15 to 30 min., allowance being made for the volume of material to be sterilized. All nutrient mediums used in the water-bacteriology laboratory can be sterilized by pressure-steam sterilization.

All mediums containing sugars should be sterilized at the minimum temperature for the minimum time to avoid any breaking down of the sugars. A breakdown of the lactose will result in false positives in the presumptive test, inasmuch as

dextrose-fermenting bacteria will produce gas. The medium should be cooled immediately upon removal from the autoclave. The tubes containing medium should be packed loosely in small wire baskets for sterilization and the center of the medium should never be more than 2.5 cm. from the outside surface of the glass or the surface of the medium. The medium should be packed in the autoclave in such a manner that the steam can circulate on the sides, top and bottom of each basket or flask.

Nutrient agar for plating may be sterilized in 200-ml. flasks containing approximately 100 ml. per flask. Each flask will contain sufficient agar for pouring 8 to 10 plates. If only 2 to 4 plates are made at a time, it will be more convenient to store nutrient agar in test tubes.

In operating a pressure cooker or autoclave, care must be exercised to free the autoclave of all air, as the presence of air decidedly reduces the effectiveness of the sterilization process. When air and steam occupy the space in the autoclave, the total pressures are the sum of the pressures exerted by the steam and the air. The temperature will correspond to the pressure of the steam. If the air is allowed to remain in an autoclave at 18 lb. pressure, 6 lb. pressure will be due to the air while 12 lb. will be due to the steam. Autoclaves should be equipped with a steam gauge and a thermometer. The pressure and the temperature should be in agreement for successful sterilization processes.

Filtration.

All liquid and nutrient agar mediums used for the growth of bacteria, wherein clarity is important, should be free from suspended particles. Dehydrated mediums have been clarified by the manufacturer so that filtration is unnecessary.

All liquid mediums are readily clarified by passing through plaited filter paper. Agar mediums may also be clarified in a similar manner, but frequently this method is far too slow to be practicable. An easily prepared and quick-acting filter is made by placing a layer of absorbent cotton between two layers of cotton gauze. This is placed in the bottom of a Büchner filter funnel. The agar passes readily, particularly if the filter is washed with hot water just prior to filtering the agar.

Adjustment of the Reaction of Mediums.

It is very important that the hydrogen-ion concentration (pH) of culture mediums be adjusted accurately. The coliform organisms show their maximum reproduction rate at pH 6.8. Although rates of reproduction fall only slightly at pH above or below this value, the variation should not be more than pH 0.3 ±. For uniformity of results, the pH value of successive batches of medium should be the same.

The pH of culture mediums may be determined either electrometrically by means of a glass electrode or colorimetrically. The former is the more accurate procedure and should be used when a pH meter is available. Directions for the use of the pH meter accompany the apparatus.

For laboratories not equipped with pH meters, the colorimetric method can be used. For a discussion of hydrogen-ion concentration see page 144. For a determination of the hydrogen-ion concentration by the colorimetric method see page 10. To adjust the pH of a medium proceed as follows:

1. Add 5 ml. of distilled water to each of two test tubes.
2. Add 5 ml. of the medium to be tested to each of the above tubes.
3. Add 0.5 ml. of bromthymol blue indicator (page 250) to one tube.
4. Add 0.05N sodium hydroxide (page 251) until the color is the same as that of the standard of the pH desired, when checked as directed on page 10. Record the amount of sodium hydroxide used. The amount of 0.05N sodium hydroxide necessary to titrate to the desired color is the amount of 1N sodium hydroxide necessary to adjust 100 ml. of medium. (The 1N sodium hydroxide may be prepared according to the method given on page 102 for 0.5N.)

Preparation of Sodium Thiosulphate Sample Bottle.

Three methods of preparing sodium thiosulfate sample bottles are presented. All three of these methods are equally effective.

Moist-heat Sterilization.

Option 1.—Dissolve 1.5 grams of sodium thiosulfate in 100 ml. of distilled water. Place 0.5 ml. of this solution in each clean

bottle. (This amount has been found sufficient to reduce residual chlorine completely in an amount up to 2.0 p.p.m. in a sample of 150 ml. of water.) Stopper and cap each bottle. Place the bottles in an autoclave and sterilize for 15 min. at a pressure of 20 lb. per square inch.

Option 2.—Place approximately 0.02 to 0.05 gram of powdered sodium thiosulfate into each clean wet bottle. The amount need not be weighed. An estimated amount on the tip of a spatula is sufficiently accurate. Sterilize the bottles as in Option 1.

Dry-heat Sterilization.

Add from 0.02 to 0.05 gram of powdered sodium thiosulfate into each clean dry bottle as in Option 2 under moist-heat sterilization. Stopper, cap and sterilize at 180°C. for 10 min. The temperature of sterilization must not approach within 5 or 10° of 220°C. as sodium thiosulfate decomposes at this temperature.

DIVISION II

PREPARATION OF NUTRIENT MEDIUMS

LIQUID MEDIUMS

Nutrient Lactose Broth.

1. Add 3 grams of beef extract (Bacto or equivalent) to 1,000 ml. of distilled water and sprinkle 5 grams peptone (Bacto or equivalent) over the surface of the liquid.
2. Place in Arnold steamer or heat to approximately 65°C. on water bath until all ingredients are dissolved. (An autoclave with open exhaust valve may be used in place of Arnold steamer.)
3. Adjust the pH to 7.0 (page 241). The final pH after sterilization should be between 6.4 and 7.0 and preferably 6.8.
4. Place in Arnold steamer for 15 to 30 min. or bring to boiling on water bath. Make up evaporation loss with distilled water if the latter procedure is used.
5. Cool and clarify by passing through filter paper or as suggested on page 240.
6. Add 0.5 per cent, by weight, of lactose, C.P.
7. Distribute in Durham fermentation tubes as follows:
   a. 30 ml. for 10-ml. water portions.
   b. 8 to 10 ml. for 1-ml. or decimal dilutions of water portions.
8. Sterilize in the autoclave at 15 lb. pressure for 15 min.

Note.—If desired, double-strength broth may be prepared for 10-ml. water portions by using double the quantities of beef extract, peptone and lactose in the above procedure. Distribute 8 to 10 ml. of double-strength broth in fermentation tubes. If 100-ml. water portions are tested, 50-ml. quantities of double-strength broth may be used.

Unless broth is in large quantities, it will be found more economical and convenient to use a dehydrated medium. (Difco).

Broth is best stored at room temperature and should be in such quantities that evaporation is not excessive before the entire batch is used. Tubes containing gas bubbles in the inserts should be discarded.

Brilliant Green Bile Lactose Broth.

1. Add 10 grams of peptone (Bacto or equivalent) to 750 ml. of distilled water.
2. Place in Arnold steamer until the peptone is dissolved.
3. Add 200 ml. of fresh ox bile, or 20 grams dehydrated ox bile dissolved in 200 ml. distilled water. (pH of bile solution should be pH 7 or higher.)
4. Adjust the reaction to pH 7.4 (page 241).
5. Add 10 grams lactose C.P. and 13.3 ml. of 0.1 per cent aqueous solution of certified brilliant green (page 251).
6. Make up to 1,000 ml. with distilled water.
7. Distribute in approximately 10-ml. amounts in Durham fermentation tubes.
8. Sterilize in the autoclave at 15 lb. pressure for 15 min.

Note.—Unless considerable quantities are needed, the dehydrated medium, Bacto 2 per cent bile brilliant green lactose broth, will be found more economical and convenient to use.

Fuchsin-Lactose Broth.

1. To 1,000 ml. of nutrient lactose broth (page 243) add 15 ml. of a 0.1 per cent solution of certified basic fuchsin (95 per cent dye content) in water.
2. Sprinkle 5 grams peptone (Bacto or equivalent) over the surface of the liquid.
3. Place in Arnold steamer or over a free flame until all ingredients are dissolved.
4. Adjust the reaction so that the final pH, after sterilization, will be 7.3 to 7.5 (page 241).
5. Add 5 grams lactose, C.P.
6. Distribute in approximately 8- to 10-ml. quantities in Durham fermentation tubes.
7. Sterilize in the autoclave at 15 lb. pressure for 15 min.

Note.—Unless considerable quantities are needed, the dehydrated medium, Bacto fuchsin lactose broth, will be found more economical and convenient.

Crystal Violet Lactose Broth.

1. Add 5 grams dipotassium phosphate ($K_2HPO_4 \cdot 3H_2O$) and 1 gram potassium dihydrogen phosphate (KH_2PO_4) to 1,000 ml. of distilled water in an enamelware pail.

2. Sprinkle 5 grams of peptone (Bacto or equivalent) over the surface of the liquid.
3. Place in Arnold steamer or heat over free flame until all ingredients are dissolved.
4. Add 5 grams lactose C.P. and 7.1 ml. of a 1 to 5,000 solution of certified crystal violet (page 251).
5. Mix and distribute in approximately 10-ml. quantities in Durham fermentation tubes.
6. Sterilize in the autoclave at 15 lb. pressure for 15 min.

Note.—Unless considerable quantities are needed, the dehydrated medium, Bacto crystal violet lactose broth, will be found more economical and convenient to use.

Citrate Medium.

1. Add 1.5 grams sodium ammonium phosphate (microcosmic salt), 1 gram potassium dihydrogen phosphate, 0.2 gram magnesium sulfate and 2.5 grams sodium citrate (crystal) to 1,000 ml. of distilled water. Mix thoroughly until all ingredients are dissolved.
2. Distribute in test tubes, 5 ml. per tube.
3. Sterilize in the autoclave at 15 lb. pressure for 15 min.

Note.—Unless considerable quantities are needed, the dehydrated medium, Bacto citrate medium, will be found more economical and convenient to use.

Eijkman Medium.

1. Add 15 grams Bacto tryptose, 4 grams of dipotassium phosphate, 1.5 grams of potassium dihydrogen phosphate and 5 grams of sodium chloride to 1,000 ml. of distilled water.
2. Heat in Arnold steamer or over free flame until all ingredients are dissolved.
3. Filter through filter paper (page 240).
4. Add 3 grams of lactose C.P.
5. Distribute in test tubes, 10 ml. per tube.
6. Sterilize in autoclave at 15 lb. pressure for 15 min.

Note.—Unless considerable quantities are needed, the dehydrated medium, Bacto Eijkman, will be found more convenient and economical to use.

Dextrose Dipotassium Phosphate Medium.

1. Add 5 grams of Bacto proteose-peptone, 5 grams dextrose C.P. and 5 grams dipotassium phosphate ($K_2HPO_4 \cdot 3H_2O$) to 800 ml. of distilled water.

2. Heat in Arnold steamer or over water bath for 20 min.
3. Filter through filter paper (page 240), cool to 20°C. and make up to 1,000 ml. with distilled water.
4. Distribute in test tubes, 5 ml. per tube.
5. Sterilize in autoclave at 15 lb. pressure for 15 min.

Note.—Unless considerable quantities are needed, the dehydrated medium, Bacto dextrose dipotassium phosphate medium, will be found more convenient and economical to use.

Tryptone Broth.

1. Sprinkle 10 grams of Bacto tryptone over surface of 1,000 ml. of distilled water in an enamelware pail.
2. Heat in Arnold steamer until the tryptone is in solution.
3. Distribute in test tubes, 5 ml. per tube.
4. Sterilize in autoclave at 15 lb. pressure for 15 min.

Note.—Unless considerable quantities are needed, the dehydrated medium, Bacto tryptone broth, will be found more convenient and economical to use.

Formate-ricinoleate Broth.

1. Add 5 grams of sodium formate and 1 gram of sodium ricinoleate to 1,000 ml. of distilled water in an enamelware pail.
2. Sprinkle 5 grams of peptone over the surface of the liquid.
3. Steam or heat over a free flame until all ingredients are dissolved.
4. Adjust the reaction so that the final pH, after sterilization, will be 7.3 to 7.5.
5. Add 5 grams of lactose.
6. Distribute in approximately 8- to 10-ml. amounts in Durham fermentation tubes.
7. Sterilize in the autoclave at 15-lb. pressure for 15 min.

Note.—Unless considerable quantities are needed, the dehydrated medium, Bacto formate-ricinoleate broth, will be found more economical to use.

SOLID MEDIUMS

Nutrient Agar.

1. Add 15 grams of stock shredded or powdered agar to 1,000 ml. distilled water.

2. Add 3 grams beef extract (Bacto or equivalent); sprinkle 5 grams of peptone (Bacto or equivalent) over surface of mixture.
3. Cover container and place in Arnold steamer until all ingredients are dissolved.
4. Adjust the reaction to pH 7 to 7.2 (page 241).
5. Place in the Arnold steamer for 30 min. Clarify as indicated on page 240.
6. Distribute approximately 100-ml. quantities in 200-ml. Erlenmeyer flasks or approximately 10-ml. quantities in test tubes.
7. Sterilize in the autoclave at 15 lb. pressure for 15 min.

Note.—Unless large quantities are used, the dehydrated medium, Bacto nutrient agar, will be found more economical and convenient to use.

Preparation of Agar Slants and Method of Seeding.

The standard nutrient agar (page 246) used for plating should be used for agar-slant cultures. Place the melted agar in test tubes, adding approximately 8 to 10 ml. per tube. After the tubes are stoppered with nonabsorbent cotton, they are sterilized in the autoclave in the same manner as nutrient agar prepared for plating purposes. After sterilization, the tubes are immediately placed on an inclined surface so the agar will solidify in a slanted position with the top of the agar at least an inch from the cotton stopper. After the agar has solidified, the tubes may be stored in the refrigerator.

The agar slants are seeded as follows:

1. Use a straight platinum needle without a loop.
2. Select a well-isolated colony of the type desired.
3. Flame the needle in an open flame until the platinum becomes a cherry red. Allow to cool.
4. Fish material from the colony by dipping the tip of the needle into the surface of the colony. Only a tiny speck of the material is necessary.
5. Flame the mouth of the agar-slant tube.
6. Streak material from the platinum needle upon the surface of the slant; be careful not to cut into the surface of the agar.

Eosin-Methylene Blue Agar.

1. Place 15 grams of clean shredded or powdered agar and 1,000 ml. of distilled water in an enamelware pail.

2. Add 2 grams of dipotassium phosphate ($K_2HPO_4 \cdot 3H_2O$).
3. Sprinkle 10 grams of peptone (Bacto or equivalent) over the surface of the mixture.
4. Cover the pail and place in Arnold steamer for 30 min. until all the ingredients are dissolved. If the ingredients are dissolved on a water bath, the loss by evaporation should be replaced by distilled water.
5. Add 10 grams of lactose, 20 ml. of a 2 per cent aqueous solution of yellowish eosin (page 251) and 20 ml. of a 0.5 per cent aqueous solution of methylene blue (page 251) and mix well.
6. Place the desired quantities in flasks, bottles or tubes and sterilize in the autoclave at 15 lb. pressure for 15 min.
7. Prepare plates by pouring 10 to 12 ml. of the sterilized medium into sterile Petri dishes. If the medium is poured hot, leave the Petri dish covers ajar until the medium has solidified. Leaving the dishes partially open lessens the amount of condensate on the agar surfaces. A number of plates may be prepared at one time and stored in the refrigerator. Plates should not be stored more than 72 hr. because of drying of the agar surface. Never expose plates to direct sunlight.

Note.—The dehydrated product (Bacto eosin-methylene blue agar) is very simple to prepare. Most laboratories prefer the dehydrated product.

Endo's Medium.

1. Add 30 grams of air-dried agar and 5 grams of beef extract (Bacto or equivalent) to 1,000 ml. of distilled water in an enamelware pail.
2. Sprinkle 10 grams of peptone (Bacto or equivalent) over the surface of the mixture. Cover the pail and place in Arnold steamer for 30 min. or until all ingredients are dissolved. If the ingredients are dissolved over water bath, the loss by evaporation should be replaced with distilled water.
3. Adjust the reaction to pH 7.4 (page 241).
4. Filter as recommended on page 240.
5. Place in 200-ml. flasks, 100 ml. to each, and sterilize at 15 lb. pressure for 15 min.

When Endo's agar plates are desired the agar is melted and the following is added to each flask:

a. 5 ml. of sterile 20 per cent lactose solution.
b. 1 ml. of a 1 per cent solution of basic fuchsin in 95 per cent alcohol (page 251).
c. Add 0.5 ml. of a 25 per cent freshly prepared solution of anhydrous sodium sulfite* (page 252).

The plates are prepared by pouring 10 to 12 ml. of the above medium into sterile Petri dishes while hot. Leave the Petri dish covers slightly ajar until the medium solidifies as this lessens the amount of condensate on the agar surface. Because this medium does not keep well after the addition of the fuchsin and sodium sulfite, the number of plates prepared should be limited to those needed for immediate use.

Tryptone Glucose Extract Agar.

Tryptone glucose extract agar is the standard plating medium for milk analysis ("Standard Methods for the Examination of Dairy Products," 8th ed., American Public Health Association, New York, N. Y., 1941). Bacteria counts are generally slightly higher than those obtained on nutrient agar but the difference on most waters is not significant.

1. Add 15 grams of air-dried agar and 3 grams of beef extract (Bacto or equivalent) to 1,000 ml. of distilled water.
2. Sprinkle 5 grams of Bacto tryptone over the surface.
3. Place in Arnold steamer or boil over free flame until all ingredients are dissolved. Make up lost weight with distilled water.
4. Adjust the reaction so that the pH reading after sterilization will be between 6.6 and 7.0.
5. Heat in Arnold steamer for 30 min. or bring to boiling over free flame. Restore lost weight with distilled water.
6. Filter through nonabsorbent cotton (page 240).
7. Add 5 grams of glucose.
8. Distribute in tubes or flasks as desired.
9. Sterilize in autoclave at 15 lb. pressure for 15 min.

Note.—The dehydrated product (Bacto tryptone glucose extract agar) is very simple to prepare. Most laboratories prefer the dehydrated product.

* The medium should be light pink while hot and almost colorless after cooling. Since this medium does not keep well after the addition of fuchsin and sodium sulfite, only enough for immediate use should be prepared.

DIVISION III

PREPARATION OF SOLUTIONS

Preparation of Dilution Water.—The water used for dilution purposes must be tap water, or biochemical oxygen-demand dilution water. Distilled water should never be used. The dilution flasks should be milk-dilution flasks with Escher rubber stoppers. If tubes are used, ¾- by 6-in. tubes are preferable. Tubes of smaller diameter are too small for proper mixing of 10-ml. quantities. The dilution flasks or tubes may be prepared by either *a* or *b* as follows:

a. Sterilize a flask of tap or biochemical oxygen-demand water by steam under pressure. Place this sterile water in sterile ¾- by 6-in. (or larger) test tubes or flasks with sterile pipettes. For convenience, 9-ml. amounts may be placed in test tubes.

b. Place tap water or biochemical oxygen-demand water in test tubes or flasks and then sterilize by steam under pressure. Sufficient excess water should be placed in the flasks or test tubes so that the desired amount is present after sterilization. If 9-ml. amounts are desired, approximately 10 ml. of water should be used. If 90- or 99-ml. amounts are desired, approximately 3 ml. of additional water should be added. The exact amount to add should be determined by testing in your own autoclave.

Bromthymol Blue Indicator. *Stock Solution.*—Grind 1 gram of bromthymol blue (dibromo thymol sulfophthalein) in 32 ml. of 0.05N sodium hydroxide (page 251). When the indicator is completely in solution, make up to 250 ml. with distilled water.

Test Solution of Indicator.—Add 9 ml. of distilled water to 1 ml. of the above stock solution. The indicator solution should be used in the ratio to the solution to be tested of 0.5 to 10 ml.

Iodine Solution for Gram's Stain.—Dissolve 3 grams of potassium iodide and 1 gram of iodine crystals in 100 ml. of distilled water.

Acetone-Ether for Gram's Stain.—Mix 25 ml. of ether and 75 ml. of acetone.

One Per Cent Methyl Violet for Gram's Stain.—Add 1 gram of methyl violet to 100 ml. of distilled water. Shake thoroughly and allow to stand at least 24 hr. Decant or filter to eliminate any precipitates. This solution is exceedingly stable and may be kept for long periods without any appreciable deterioration.

Sodium Bicarbonate Solution for Gram's Stain.—Dissolve 5 grams of sodium bicarbonate in 100 ml. of distilled water.

Aqueous Yellowish Eosin for Eosin-Methylene Blue Medium.—Dissolve 2 grams of yellowish eosin in 100 ml. of distilled water.

Aqueous Methylene Blue for Eosin-Methylene Blue Medium.—Dissolve 0.5 gram of certified methylene blue in 100 ml. of distilled water.

Aqueous Brilliant Green Solution for Brilliant Green Bile Broth. Dissolve 0.1 gram of certified brilliant green in 100 ml. of distilled water. Solutions should not be kept longer than 1 month.

Sodium Hydroxide (0.05N).—To 5 ml. of a 1N sodium hydroxide solution add 95 ml. of distilled water.

Two Per Cent Aqueous Safranin O for Gram's Stain.—Dissolve 2 grams certified safranin O in 100 ml. of distilled water. Shake thoroughly and allow to stand at least 24 hr. Decant or filter to eliminate any precipitates. This solution is stable and may be kept for long periods of time.

Fuchsin Solution for Endo's Medium.—Add 1 gm. of certified basic fuchsin (85 to 90 per cent actual dye content) to 99 ml. of 95 per cent ethyl alcohol. This solution should preferably be prepared just prior to adding to the medium.

Crystal Violet Solution for Crystal Violet Broth.—Place 1 gram of certified crystal violet in a glass-stoppered bottle of 150-ml. capacity and add 50 ml. of 95 per cent ethyl alcohol. Shake thoroughly and then add 50 ml. of distilled water. Shake well again until all of the dye is dissolved. Pipette 20 ml. of this 1 per cent solution into a 1-liter volumetric flask. Add sufficient water to make 1 liter. This gives a 1 to 5,000 solution of crystal violet.

Fuchsin Solution for Fuchsin Lactose Broth.—Add 0.1 gram certified basic fuchsin to 100 ml. of distilled water. Shake thoroughly and allow to stand overnight.

Malachite Green Solution for Spore Stain.—Dissolve 5 grams of malachite green in 100 ml. of distilled water. Shake thoroughtly and allow to stand overnight.

Safranin Solution for Spore Stain.—Dissolve 0.5 gram certified safranin O in 100 ml. of distilled water. Shake thoroughly and allow to stand overnight.

Sodium Sulfite Solution for Endo's Medium.—Add 5 grams anhydrous sodium sulfite to 20 ml. of distilled water. Heat to boiling temperature. Always prepare fresh solution.

SECTION IV

GENERAL DISCUSSION OF BACTERIOLOGY

PURPOSE OF BACTERIOLOGICAL ANALYSIS

Coliform Group.

The bacteriological analysis of water is for one purpose only, namely, to determine the potability of water. Diseases of intestinal origin, typhoid fever and dysentery particularly, have been and are transmitted by sewage-polluted waters. Before the introduction of clean water the typhoid death rate was very high. For 1900 the rate for the United States was 35.9 while in 1941, with relatively safe water in use, the death rate had fallen to 0.8 per 100,000 population.

The measuring stick used for determining the safety of the water is the bacteriological analysis. The minute number of disease organisms in a water supply makes their isolation unreliable if not impossible. Further, the methods of isolation are difficult and not adapted to routine laboratory procedure. It has been necessary to test for organisms associated with the disease producers that are present in polluted waters in large numbers, that are more easily isolated and that are easily identified.

There are three groups of bacteria present in the intestinal tract of man and animal in abundance, namely the coliform, the anaerobic lactose-fermenting sporeformers and the fecal streptococci. Of these three groups, the coliform organisms are more closely related to the intestinal pathogens (typhoid, dysentery and paratyphoid organisms) and hence are affected by storage, sedimentation, chlorination and other natural or induced processes of purification to approximately the same degree. Thus the absence of the coliform organisms would indicate also the absence of the intestinal pathogens. The test for the coliform group thus is an indirect one. The test must be

quantitative to measure the density of the organisms in the water. Tests are occasionally made for the anaerobic spore-forming bacilli and the streptococci but their use is so infrequent that a discussion of these groups is not included.

The coliform group of bacteria includes two genera of bacteria, the Escherichia and the Aerobacter. "Standard Methods for the Examination of Water and Sewage," 8th ed., defines this group as "all aerobic and faculative Gram-negative nonspore-forming bacilli which ferment lactose with gas production." Many synonyms have appeared in the literature, such as the coli-aerogenes group, Escherichia-Aerobacter group, *B. coli* and *Bact. coli*. The preferred name is the "coliform group."

For purposes of identification the coliform group is only checked as to genera *Escherichia* and *Aerobacter*. For purposes of measuring the potability of water the identification of the group can be limited to the following characteristics:

1. (*a*) Ability to ferment lactose with gas formation in nutrient lactose broth, followed by
   (*b*) Ability to ferment lactose in brilliant green bile lactose broth or,
2. (*a*) Ability to ferment lactose with gas formation in nutrient lactose broth, followed by
   (*b*) Ability to grow on surface of Endo's medium or eosin-methylene blue agar. The organisms isolated from these mediums should ferment lactose in nutrient lactose broth. The organism should be Gram negative and not form spores.

For routine sanitary water analysis no distinction is made between the *Escherichia* and *Aerobacter* genera.

Gas Formation.

The first step in the bacteriological analysis is the development of the coliform organisms in standard lactose broth. The coliform group ferments lactose with the production of gas. The production of gas from lactose is a presumptive test for the group but not a definitely positive reaction because a few other bacteria, not necessarily of sanitary significance, may also produce the same reaction. The most common bacteria, other than the coliform group, which produce gas are the sporeformers, both

anaerobic and aerobic. Sometimes a synergism may cause gas from lactose. One organism splits the lactose into glucose and a second organism, unable to attack lactose, ferments the the glucose with gas formation. In heavily polluted water these organisms are far in the minority as compared with the coliform group and in such waters the lactose-broth fermentation may be considered as a completed test. The sporeformers are likely to be the cause of gas formation in finished waters, swimming-pool waters and chlorinated sewage effluents owing to the resistance of the spores to chlorination.

The amount of gas produced in a 48-hr. period of incubation at 37°C. has no particular significance. Some strains of the coliform organisms produce only slight amounts of gas. As a matter of fact, some strains do not produce gas in 48-hr. incubation. These organisms are missed in the regular standard procedure. Any amount of gas should be considered a positive presumptive test if the gas appears within 48-hr. incubation at 37°C.

Many attempts have been made to introduce some agent into lactose broth that would eliminate these spurious tests. Many chemicals have been found that will eliminate these undesirable microorganisms and thus produce a negative test for these latter organisms. In addition, in certain strains of the coliform group the inhibitory agents suppress the ability to produce gas. Recently a new series of inhibitory agents have been found that apparently have no objectionable action on the coliform group, for example, sodium lauryl sulfate. It is possible such a product may ultimately be added to the lactose broth so that the appearance of gas in the primary lactose broth will constitute a completed test in itself.

Aerobic Growth.

The definition of the coliform group states that they grow aerobically on solid mediums. To demonstrate this property, smears are made on eosin-methylene blue agar or Endo's medium from the tubes showing gas. The smears should be made so that discrete colonies are produced. It must be remembered that the growth in the lactose broth is due to many species of bacteria in addition to the coliform organisms. Unless dis-

crete colonies are formed, it is impossible to make isolations representing pure cultures because the confluent growth is a mixed population. The appearance of the confluent growth as indicating coliform organisms may be very misleading owing to the multiplicity of reactions induced by the different species of bacteria that may be present.

By smearing the surface of the agar plate, the coliform group is able to grow but the anaerobic sporeformers are eliminated as they are unable to grow aerobically. The dyes in the medium also act as a deterrent to many of the undesirable bacteria. To distinguish the coliform organisms from other organisms that might grow on the agar plate, the use of special mediums, like eosin-methylene blue agar or Endo's medium, gives the coliform colonies a distinctive appearance that aids in their identification. A characteristically typical colony generally proves to be a member of the coliform group. In the absence of typical colonies, several different atypical colonies should always be fished and checked further.

Gram-negative, Nonsporeforming Bacilli.

To make sure that either typical or atypical colonies are members of the coliform group, the colonies fished (use care to pick discrete colonies that are well isolated) are planted into lactose broth and on an agar slant. If the organisms produce gas in the fermentation tube, then the corresponding agar-slant culture should be examined for spores and Gram's reaction These tests eliminate the aerobic, lactose-fermenting, Gram-positive sporeformers. The only organisms that successfully carry through these tests are the members of the coliform group.

In the routine laboratory where the analyst has many duties besides making the bacteriological tests, the procedure as outlined above, although a satisfactory test, requires too much time. Also the technique involved requires considerable skill and experience which the analyst, unfortunately, frequently lacks. For these people and others who need a rapid test, another procedure or modification is often used. In this procedure the presumptive test is made as usual but instead of smearing eosin-methylene blue agar or Endo's medium, plantings are made into one of the following mediums: brilliant green bile lactose broth,

fuchsin broth, formate-ricinoleate broth or crystal violet broth. If possible, plantings should be made from the presumptive lactose-broth tubes as soon as gas appears. The plantings are incubated 48 hr. at 37°C. If gas appears during this period of incubation, the test is reported as positive.

The four mediums were originally offered on the basis that certain mediums might be better for certain types of water. Subsequently, it has been found that brilliant green bile broth serves best, hence it is suggested that this medium be used.

All these mediums contain inhibitory agents that prevent the growth of organisms causing spurious tests. Theoretically, they are not inhibitory to the coliform group. This is very largely true when the selective medium is planted with young actively growing bacteria such as would be obtained from a 24-hr. or younger lactose-broth culture.

The Colon Index.

When individual samples of water are examined it is not necessary to express the number of the coliform group present. Under such conditions the amount of water tested and the presence or absence of the coliform group should be stated. When samples of water from the same supply are routinely tested this method of expressing the results becomes cumbersome. Expressing the number of coliform organisms present is more convenient. The colon index is the number of these organisms per 100 ml. of water.

Two methods of determining the number of organisms are used. The Phelps index, or reciprocal method of interpreting the number of organisms in a decimal dilution system, is designed primarily for a single-tube dilution series. Although this method is not sound mathematically, it is a comparatively useful method. The other method is the Most Probable Number (M.P.N.). The value obtained by this method is defined by Hoskins and Butterfield as "that bacterial density, which if it had been actually present in the sample under examination, would, more frequently than any other, have given the observed analytical results."

In routine tests, most laboratories require the daily computation of the colon index. Either the Phelps index method or the

M.P.N. method is used. Tables 10 and 11 are presented giving the most common setups made in the water laboratory.

For finished water, it is recommended (page 224) that five 10-ml. and one 1-ml. portions be planted. Table 10, below, shows the colon index computed by both the Phelps and M.P.N.

TABLE 10.—TEST RESULTS FOR POTABLE WATERS AND COMPUTED COLON INDEX

Portions planted,* ml.							Computed colon index	
10	10	10	10	10	1	0.1	Phelps	M.P.N.
−	−	−	−	−	−	+	...	2.0
−	−	−	−	−	+	−	2	2.0
−	−	−	−	−	+	+	...	4.0
+	−	−	−	−	−	−	2	2.2
+	−	−	−	−	−	+	...	4.4
+	−	−	−	−	+	−	4	4.4
+	−	−	−	−	+	+	...	6.7
+	+	−	−	−	−	−	4	5.0
+	+	−	−	−	−	+	...	7.5
+	+	−	−	−	+	−	6	7.6
+	+	−	−	−	+	+	...	10.0
+	+	+	−	−	−	−	6	8.8
+	+	+	−	−	−	+	...	12.0
+	+	+	−	−	+	−	8	12.0
+	+	+	−	−	+	+	...	16.0
+	+	+	+	−	−	−	8	15.0
+	+	+	+	−	−	+	...	20.0
+	+	+	+	−	+	−	10	21.0
+	+	+	+	−	+	+	...	27.0
+	+	+	+	+	−	−	10	38.0
+	+	+	+	+	−	+	...	96.0
+	+	+	+	+	+	−	100	240.0

* When only five 10-ml. and one 1-ml. dilutions are made from a sample, indices should be taken from those lines in which a negative test is obtained in the 1-ml. column.

methods. It is assumed that a 0.1-ml. portion would be negative. If the coliform frequency is such that a 0.1-ml. portion is positive, decimal dilutions should be used. Actually a finished water should have a colon index of 1 or less, so a 0.01-ml. portion is not necessary.

If the same number of samples are tested daily, both the Phelps and the M.P.N. monthly indices may be obtained by

dividing the sum of the daily averages by the number of days. If the number varies from day to day, the sum of the total Phelps

TABLE 11.—TEST RESULTS FOR UNPOTABLE WATERS AND THE COMPUTED COLON INDEX

Portions planted, ml.							Computed colon index	
10	1	0.1	0.01	0.001	0.0001	0.00001	Phelps	M.P.N.
−	−	+						9.0
−	+	−					10	9.4
−	+	+					100	19.0
+	−	−					10	23.0
+	−	+					100	95.0
+	+	−					100	240.0
	−	−	+					90.0
	−	+	−				100	94.0
	−	+	+				1,000	190.0
	+	−	−				100	230.0
	+	−	+				1,000	950.0
	+	+	−				1,000	2,400.0
		−	−	+				900.0
		−	+	−			1,000	940.0
		−	+	+			10,000	1,900.0
		+	−	−			1,000	2,300.0
		+	−	+			10,000	9,500.0
		+	+	−			10,000	24,000.0
			−	−	+			9,000.0
			−	+	−		10,000	9,400.0
			−	+	+		100,000	19,000.0
			+	−	−		10,000	23,000.0
			+	−	+		100,000	95,000.0
			+	+	−		100,000	240,000.0
				−	−	+		90,000.0
				−	+	−	100,000	94,000.0
				−	+	+	1,000,000	190,000.0
				+	−	−	100,000	230,000.0
				+	−	+	1,000,000	950,000.0
				+	+	−	100,000	2,400,000.0

or M.P.N. indices should be divided by the number of samples tested.

For raw waters it is recommended that a minimum of three-decimal dilutions be made, so that the highest dilution will

give negative results. Table 11 gives the Phelps and the M.P.N. indices for different results from a number of three-dilution plantings.

When more than three dilutions are made, the M.P.N. is determined practically by the tubes of the dilutions in which the change is from positive to negative. In the five-dilution series given below the three dilutions used for calculating the M.P.N. index are enclosed in parentheses.

+(+ + −)−	+(+ − +)−	++(+ + −)
M.P.N.	M.P.N.	M.P.N.

The Counting of an Agar Plate.

The 37°C. agar plates planted for total count should always be counted at the end of 24-hr. incubation. The 24-hr. count is standard practice. An incubation longer than this period will give a higher count as slowly growing bacteria will gradually develop visible colonies. Because the count is not a total count in the sense of representing all the bacteria present, and because it is made for comparative purposes, it is necessary that all the steps in the procedure be made exactly in the same manner.

For counting, select the dilution plate that contains preferably between 30 and 300 colonies. Plates containing a number of colonies in this range give the most accurate count of the number of bacteria actually present. If a plate contains less than 30 colonies, the colonies entering as contamination become significant in the total number present. If more than 300 colonies are present, the plate becomes overpopulated and many bacteria may be suppressed, thus preventing the development of many colonies. Counts made from overcrowded plates generally show a count much lower than the number actually present.

Duplicate 1-ml. portions of all potable waters are plated to be sure that one satisfactory plate is obtained. If one or more colonies are spread over the plate surface, the plate should be discarded. If both plates are satisfactory, the counts may be averaged.

The counts should be made with a lens of 2.5 diameters magnification. All colonies, irrespective of size, visible under

this magnification should be recorded. If the 1-ml. portion shows less than 30 colonies, this count should be recorded as it is impracticable to plate larger amounts of water.

A Wolfhugel or Jeffers counting plate will be found of value in making the counts. There are on the market several good counting chambers designed for this purpose. The use of a hand tally is also an aid in making the counts.

The bacteria counts should always be recorded in round numbers. As the probable error is extremely high, the recording of the actual number found is sometimes a fictitious accuracy. The number should be recorded as listed in the accompanying table:

From	1 to	50 record as found	
From	51 to	100 record to the nearest	5
From	101 to	250 record to the nearest	10
From	251 to	500 record to the nearest	25
From	501 to	1,000 record to the nearest	50
From	1,001 to	10,000 record to the nearest	100
From	10,001 to	50,000 record to the nearest	500
From	50,001 to	100,000 record to the nearest	1,000
From	100,001 to	500,000 record to the nearest	10,000
From	500,001 to	1,000,000 record to the nearest	50,000
From	1,000,001 to	10,000,000 record to the nearest	100,000

Typing the Coliform Group Organisms.

For purposes of differentiation of coliform organisms as pertains to their source, three types of organisms are generally recognized, *Escherichia*, *Aerobacter* and the so-called *Intermediates*. The *Escherichia*, colon bacilli, are found primarily in the intestinal tract of man and animal. The *Intermediates* are generally soil organisms but also may be found occasionally in the intestines. Because the *Escherichia* are found in the intestines of man in extremely large numbers, they are of direct sanitary significance when found in water. The *Aerobacter* and the *Intermediates* are primarily soil organisms, but, as they have been found occasionally in the intestines, they are of problematical sanitary significance. Many investigators, including the authors, feel that, owing to their questionable character, these organisms should be considered equally as significant as the *Escherichia*. There may be instances where a safe water is

condemned because of the presence of these organisms, but, in the majority of cases, waters containing the *Aerobacter* and the *Intermediates* will also at times carry the *Escherichia* and thus be dangerous. Only when a large number of samples tested over a period of time show the *Aerobacter* or *Intermediates* and never the *Escherichia* should the water be considered free of fecal pollution. A water should never be considered as safe when the *Aerobacter* or *Intermediates* are found from the testing of only a few samples.

If the analyst wishes to identify the type of coliform organisms present in a water, the simplest practical procedure consists of determining the Voges Proskauer reaction, production of indol, growth in citrate medium and the Eijkman test. These four characteristics provide a rapid and easy means of classification. A classification prepared by Stuart et al.* follows:

	Indol	Voges Proskauer	Growth on citrate	Eijkman reaction	Probable origin
Escherichia coli					
Variety I.....	+	−	−	+	Fecal
Variety II.....	−	−	−	+	Fecal
Intermediate					
Variety I.....	−	−	+	−	Nonfecal
Variety II.....	+	−	+	−	Nonfecal
Aerobacter aerogenes					
Variety I.....	−	+	±	−	Nonfecal
Variety II.....	+	+	+	+	Nonfecal

The Procedure of Identification.

Isolation of Pure Culture.

Option A (page 227) should be followed in testing the water. Extreme care should be exercised in smearing the eosin-methylene blue agar or Endo's agar so as to obtain discrete colonies. A whole plate should be used for each smear and the entire surface should be smeared. The colony selected should be well isolated, that is, it should be spaced approximately 1 cm. from adjacent

* Stuart, C. A., Alice Zimmerman, Muriel Baker and Robert Rustigan, Eijkman Relationships of the Coliform and Related Bacteria, *Journal of Bacteriology*, vol. 43, page 587, 1942.

colonies. The colony should be examined with a lens of 2.5 diameters magnification to be sure the colony does not represent two merged colonies. The organisms from the colony selected should ferment lactose and they should be Gram negative and nonsporeforming.

Mediums Seeded.

The agar slant should be used for seeding the following mediums:

1. Dextrose dipotassium phosphate broth.
2. Tryptose broth.
3. Citrate medium.
4. Eijkman's medium.

Testing.

Voges Proskauer Reaction.—Seed dextrose dipotassium phosphate broth (page 247) from 24-hr. agar-slant culture. Incubate at 30°C. for 24 to 48 hr.

To the 5-ml. culture add 3 ml. of alpha-naphthol reagent and 1 ml. of 40 per cent sodium hydroxide. A development of a crimson to a ruby-red color in from 2 to 4 hr. after adding reagents is a positive test. Shaking speeds the reaction. The reaction should be read not later than 4 hr.

Indol Test.—Seed tryptone broth (page 247) from a 24-hr. agar-slant culture. Incubate at 37°C. for 24 hr.

Add 0.2 to 0.3 ml. of amyl alcohol indol reagent. Shake. After a minute or more a dark red ring at the surface indicates a positive indol test.

Citrate Test.—Seed the citrate medium (page 245) lightly from a 24-hr. agar-slant culture. Incubate at 37°C. for 72 to 96 hr.

Turbidity of the medium indicates a positive test. *Escherichia sp.* cannot utilize sodium citrate as a source of energy and therefore fail to grow. *Aerobacter sp.* can utilize this compound as a source of energy and accordingly grow abundantly.

Eijkman Test.—Seed Eijkman medium (page 245) from a 24-hr. agar-slant culture. Incubate immediately in a water bath at 45.5°C. ± 0.5. The surface of the medium should be at least 1 in. below the surface of the bath. Incubate for 96 hr. Gas production indicates a positive test.

Escherichia sp. of fecal origin produce a gas fermentation in this medium at 45.5°C., whereas *Aerobacter sp.* and the *Intermediates* either fail to grow or, if they grow, fail to produce gas. There are a few exceptions.

There are a number of other tests, such as the methyl red test, used for separating the three types of the coliform group, but their use is more apt to confuse the picture rather than aid in differentiation.

THE TOTAL BACTERIA COUNT FOR MEASURING CONTAMINATION

Occasionally it is desired to check for the presence of any bacteria that may be present rather than for sewage contamination. Instances of this type are found in the checking of supposedly sterile distilled water in hospitals, the examination of paper used for food containers, examination of finished paper food containers, etc. For such purposes, nutrient agar (page 246) does not serve fully, as many bacteria fail to grow on this medium. A new medium, tryptone glucose extract agar (page 249), standard medium for milk analysis, serves better. Maximum growth will be obtained by incubating at 20°C. for 5 days or at 32°C. for 48 hr. This medium can also be substituted for nutrient agar (page 246) for determining the bacterial contents of water and sewage.

TABLE 12.—SUPPLEMENTAL LIST OF EQUIPMENT NEEDED IN A CHEMICAL LABORATORY FOR BACTERIOLOGICAL ANALYSES

Apparatus	Quantity
Autoclave or pressure cooker	1
Bottles, staining, Barnes dropping	6
Cotton, nonabsorbent	5 lb.
Counter, hand tally	1
Quebec colony counter	1
Dilution bottles, milk, A.P.H.A	48
Petri dishes, 100 by 15 mm	48
Dish box, Petri, with rack	2
Fermentation tubes, Durham	144
Flasks, Erlenmeyer 250-ml	12
Funnel, Buchner 6 to 10 in	1
Hydrogen-ion apparatus with color standard 6.0 to 7.6	1
pH meter (optional)	1
Incubator	1
Microscope—student model with oil-immersion objective	1
Microscope slides, 3 by 1 in	144
Needles, inoculating—23 B. & S. gauge	3
Oven, hot-air, 18 by 14 by 14 in	1
Pipettes, 1-ml. graduated in 0.1	48
Pipettes, 10-ml. graduated in 0.1	48
Pipette box	2
Refrigerator, 6 cu.ft. minimum	1
Escher stoppers (for dilution bottles)	48
Test tubes—⅝ by 6 in., nonlipped	288
Test-tube baskets, 6 by 6 by 6 in	6
Thermometers, 10 to 250°C	2
Thermometers, 10 to 110°C	2

Chemicals	Quantity
Acetone	1 lb.
Agar-agar	5 lb.
Bacto-peptone	2 lb.
Basic fuchsin, certified (optional)	25 gm.
Bacto-beef extract	1 lb.
Brilliant green, certified	10 gr.
Bromthymol blue	10 gr.
Cedar oil immersion	25 gr.
Crystal violet, certified (optional)	10 gr.
Dextrose, C.P	½ lb.
Eosin, yellowish, certified	10 gr.
Methyl violet 2B, certified	10 gr.
Methylene blue, certified, bacteriological	10 gr.
Malachite green	10 gr.
Ethyl alcohol, 95 per cent	1 gal.

TABLE 12.—SUPPLEMENTAL LIST OF EQUIPMENT NEEDED IN A CHEMICAL LABORATORY FOR BACTERIOLOGICAL ANALYSES.—(*Continued*)

Iodine, C.P	¼ lb.
Potassium dihydrogen phosphate	½ lb.
Lactose, C.P	½ lb.
Dipotassium phosphate	½ lb.
Potassium iodide	½ lb.
Potassium or sodium dichromate	5 lb.
Sodium chloride, C.P	1 lb.
Sodium citrate, C.P. (optional)	½ lb.
Sodium ricinoleate (optional)	¼ lb.
Sodium formate, C.P. (optional)	¼ lb.
Sodium thiosulfate, C.P	1 lb.
Safranin O, certified	10 gr.
Sodium sulfate anhydrous (optional)	25 gr.
Sulfuric acid, commercial	5 lb.
Dehydrated Mediums	Quantity
Bacto nutrient lactose broth	2 lb.
Bacto nutrient agar	2 lb.
Bacto 2 per cent bile brilliant green broth	1 lb.
Bacto fuchsin broth (optional)	1 lb.
Bacto crystal violet broth (optional)	1 lb.
Bacto formate-ricinoleate broth (optional)	1 lb.
Bacto citrate medium (optional)	½ lb.
Bacto Eijkman broth (optional)	½ lb.
Bacto tryptose broth (optional)	½ lb.
Bacto dextrose dipotassium phosphate medium (optional)	½ lb.
Bacto eosin-methylene blue agar	1 lb.
Bacto Endo's medium (optional)	½ lb.
Bacto tryptose glucose extract agar (optional)	½ lb.

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